THE EFFECT OF OXYGEN ON THE PRODUCTION BY FAST NEUTRONS OF CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES[1]

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THE studies of THODAY and READ (1947) using root-tip cells of *Vicia faba* demonstrated that oxygen has a marked effect in increasing the frequency of X-ray-induced chromosome aberrations. These investigators later showed (1949) that when alpha rays are used, the oxygen effect is much less, since the yield of aberrations with oxygen present was only slightly greater than in its absence. In experiments on the oxygen effect using Tradescantia microspore chromosomes (GILES and RILEY 1949, 1950; GILES and BEATTY 1950), a pronounced effect of oxygen on X-ray-induced rearrangements has also been demonstrated. Preliminary studies of CONGER (unpublished) further indicate that there is little or no oxygen effect when aberrations are induced in mature pollen grains (exposed in air as compared with nitrogen) by alpha rays, thus agreeing with the observations of THODAY and READ (1949). In view of these results it seemed of interest to investigate the oxygen effect when fast neutrons are used to produce aberrations in Tradescantia, since this radiation is intermediate in effectiveness with respect to chromosome break production between X-rays and alpha particles (cf. KOTVAL and GRAY 1947).

METHODS

Inflorescences of *Tradescantia paludosa* (clone 5 of SAX) were exposed to fast neutrons produced from uranium fission in the Oak Ridge nuclear reactor in a manner generally similar to that used previously (GILES and CONGER 1950). However, in order to control the composition of the atmosphere in which the microspores were irradiated, the inflorescences were placed on a thin perforated platform inside a special airtight lucite exposure chamber from which air could be removed by evacuation and into which either pure oxygen, pure helium, or pure nitrogen could be introduced. Irradiations with fast neutrons were then performed with the inflorescences in the appropriate gas inside this chamber. Both prior to and following irradiations the intensity of the fast neutron beam in n units was determined by a series of measurements utilizing a 100 r Victoreen thimble ionization chamber placed inside the lucite container in the same position as that occupied by the inflorescences. The

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average fast neutron intensity during these exposures was 2.0 n per minute. For these measurements, one of the same Victoreen thimble chambers used in previous experiments was employed. This chamber differed by only 5 percent in comparative exposures with the thimble used as the standard in earlier experiments (GILES and CONGER 1950). This same thimble chamber, or one having essentially the same correction factor, was used in the earlier X-ray exposures (GILES and RILEY 1949, 1950). The dosage for each exposure was calculated by multiplying the total exposure time in minutes by the constant intensity of 2 n per minute. Irradiations were carried out at room temperature (*ca.* 22°C).

Most of the cytological observations were made of chromosome aberration types present in cells on slides made on the fourth and fifth days following irradiation. However, more limited observations were also made of chromatid aberration types present in microspores examined 24 hours after exposure.

RESULTS AND DISCUSSION

The data on the frequencies of chromosome aberration types are presented in table 1. In figure 1 are plotted the relationships between neutron dose and frequencies of interchanges (dicentric and centric rings) in oxygen and in nitrogen, plus two points obtained in helium. To this figure have been added for comparison the results obtained in a previous experiment with fast neutrons in which the exposure was made in air (GILES and CONGER 1950). The data on chromatid aberrations, based on observations at two dosage levels only, are presented in table 2 and in figure 2.

From these data the following conclusions may be drawn: (1) In the absence of oxygen, the frequencies of all types of aberrations (in both chromo-

TABLE 1

Frequencies of chromosomal interchanges (dicentrics and centric rings) and interstitial deletions (I.D.) induced by fast neutrons in Tradescantia microspores irradiated in atmospheres of oxygen, nitrogen, or helium (Exp. ORFN-5).

Dose (n)	No. of cells	Dicentrics	Centric rings	Total	Interchanges per cell	I.D.	I.D. per cell
Irradiated in oxygen							
5	800	99	42	141	0.18 ± 0.015	189	0.23 ± 0.017
10	700	144	65	209	0.30 ± 0.02	243	0.35 ± 0.02
20	350	159	68	227	0.65 ± 0.02	283	0.81 ± 0.05
30	350	228	84	312	0.89 ± 0.05	402	1.14 ± 0.06
Irradiated in nitrogen							
5	700	55	19	74	0.11 ± 0.01	94	0.13 ± 0.01
10	750	98	41	139	0.185 ± 0.016	164	0.22 ± 0.02
20	500	155	56	211	0.42 ± 0.03	295	0.59 ± 0.03
30	300	137	56	193	0.64 ± 0.046	238	0.79 ± 0.05
Irradiated in helium							
10	650	94	43	137	0.21 ± 0.02	140	0.215 ± 0.02
30	50	25	6	31	0.62 ± 0.11	44	0.88 ± 0.13

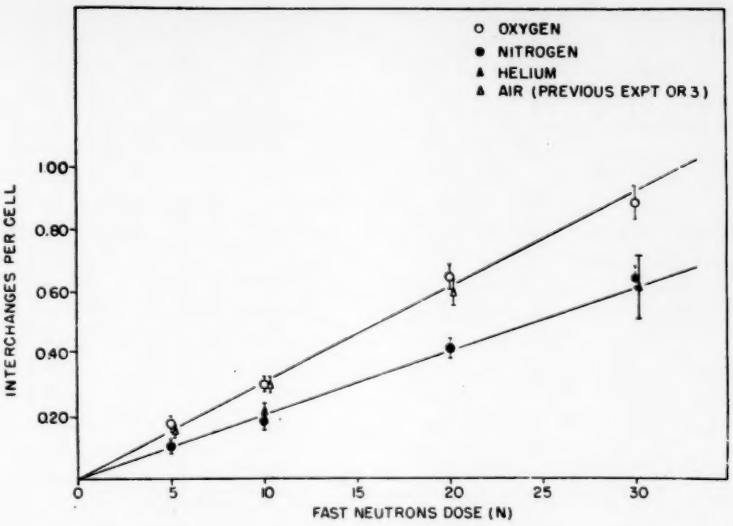

FIGURE 1.—Relation between dosage of fast neutrons and frequency of chromosomal interchanges induced in Tradescantia microspores irradiated in oxygen, air, nitrogen, or helium. The data for air were obtained in a previous experiment (ORFN-3).

some and chromatid categories) are reduced when equal dosages of neutrons are compared. (2) No significant difference is found between chromosome interchange frequencies (the only aberration type for which data are available) when neutron exposures in oxygen and in air are compared on the basis of these results and of data obtained in earlier experiments. (3) There is a linear

TABLE 2

Frequencies of chromatid aberration types induced by fast neutrons in Tradescantia microspores irradiated in atmospheres of oxygen, nitrogen, or helium (Exp. ORFN-5).

Dose (n)	No. of cells	Chromatid breaks per cell	Isochromatid breaks per cell	Exchanges per cell
		Irradiated in oxygen		
5	200	0.35 ± 0.04	0.98 ± 0.07	0.28 ± 0.04
10	200	0.77 ± 0.06	1.725 ± 0.09	0.46 ± 0.05
		Irradiated in nitrogen		
5	200	0.30 ± 0.04	0.62 ± 0.056	0.105 ± 0.02
10	200	0.58 ± 0.05	1.31 ± 0.08	0.21 ± 0.03
		Irradiated in helium		
10	200	0.58 ± 0.05	1.28 ± 0.08	0.23 ± 0.03

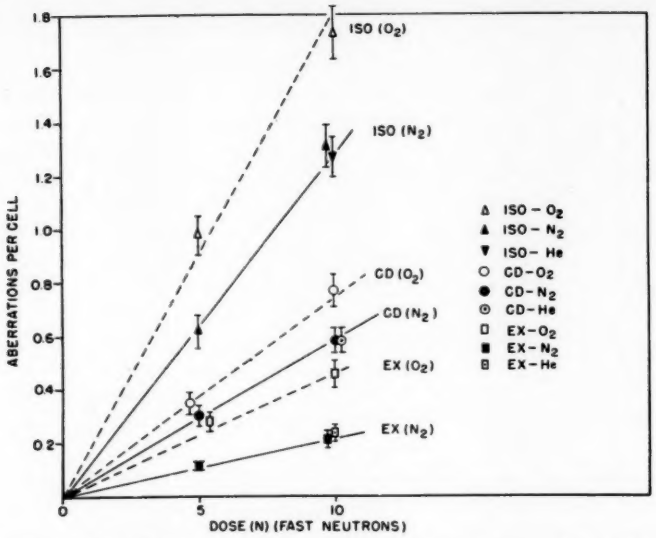

FIGURE 2.—Relation between dosage of fast neutrons and frequencies of chromatid aberration types induced in Tradescantia microspores irradiated in oxygen, in nitrogen, and in helium (Exp. ORFN-5).

relationship between neutron dose and aberration frequency for all types of aberrations (including both chromosome and chromatid interchanges) when buds are irradiated either in the presence or in the absence of oxygen.

It is clear from these results that oxygen has the effect of increasing the frequency of aberrations induced by fast neutrons. However, when a quantitative comparison is made of the present results with those obtained in experi-

TABLE 3

Comparison of the relative magnitude of the oxygen effect on chromosome aberration frequencies induced in Tradescantia by different radiations.

Radiation	Dose ratios for aberrations[3] nitrogen/oxygen				
	Chromatid breaks	Isochrom. breaks	Chromatid plus isochrom. breaks	Chromatid exchanges	Chromosome exchanges
Alpha particles	....	1.0[1]	....	1.0[1]	....
Fast neutrons	1.2	1.4	1.3	2.3	1.5
X-rays	1.4	2.6	2.0	(ca 2.5)[2]	(ca 2.5–3.5)[2]

[1]Based on preliminary data of CONGER (unpublished) obtained from irradiation of mature pollen grains in air and in nitrogen.

[2]For a further discussion of these values, see text.

[3]The dose ratio (N_2/O_2) = the ratio between a radiation dose in nitrogen and one in oxygen producing equal aberration frequencies and, consequently, is a direct measure of any effect of oxygen in increasing aberration production.

ments with X-rays (GILES and RILEY 1949, 1950; GILES and BEATTY 1950; RILEY, GILES and BEATTY 1952), it is apparent that the magnitude of the oxygen effect with fast neutrons is considerably less than it is with X-rays.

The most direct and satisfactory comparisons can be made utilizing aberrations exhibiting linear relationships with dose for both X-rays and neutrons when irradiated in oxygen or in its absence. These are the chromatid and isochromatid break types. The individual as well as the combined dose ratios for chromatid and isochromatid breaks for both X-rays and fast neutrons are shown in table 3. The most reliable cytological observations are probably for isochromatid types, and these data can also be compared directly with those of CONGER (unpublished) for alpha particles. It appears that the relation of type of radiation to the magnitude of the oxygen effect is, in increasing order, alpha particles (none), neutrons, and X-rays.

Comparison of exchange break aberrations are not as easy to make since these types show a linear relationship with dose for neutrons, but approach a dosage-square relationship with X-rays (depending on the intensity of the radiation). Unless the exponents of the comparative X-ray dosage curves obtained in oxygen and in nitrogen are equal, the value of the dose ratio (the ratio of doses which produce equal effects) will depend on the dose. In the case of X-ray-induced chromosome interchanges the evidence indicates that the exponents are different (GILES and RILEY 1950). However, a rough comparison over the X-ray-dose range used can be made and this gives nitrogen-oxygen dose-ratio values ranging from *ca.* 2.5 to *ca.* 3.5. When these values are compared with the fast neutron nitrogen-oxygen dose ratio for interchanges, obtained over a comparable range in terms of biological effect (aberration frequency per cell), again the X-ray values are higher than the neutron value of 1.5. Similar comparisons can be made for chromatid exchanges. Here the exponents of the curves obtained in oxygen and in nitrogen turned out to be essentially the same (RILEY, GILES and BEATTY 1952), and the value of the nitrogen-oxygen dose ratio is approximately 2.5. This value can be compared with the nitrogen-oxygen dose ratio for chromatid exchanges with neutrons of 2.3 and with alpha particles of approximately 1 (table 3). Despite the variations which exist in the nitrogen-oxygen dose ratios for the different aberration types within both the X-ray and neutron experiments, it is clear that in all comparisons the oxygen effect is less pronounced with neutrons than it is with X-rays. The possible significance of these variations is discussed elsewhere (RILEY, GILES and BEATTY 1952).

On the basis of the data presented in table 3, it appears that the oxygen effect with fast neutrons is intermediate in magnitude between that found with X-rays and alpha particles for all types of aberrations for which data are available. Further, it is evident that there is an inverse relationship between the specific ionization (ionization density) produced by a given type of radiation and the magnitude of the oxygen effect on chromosome aberration production.

The original results with Vicia comparing X-rays and alpha particles were

interpreted as indicating that hydrogen peroxide is responsible for chromosome aberration production, since the yield of this substance in water irradiated with alpha particles is independent of the oxygen concentration, but increases markedly with increasing oxygen concentration in water irradiated with X-rays. Other parallelisms between hydrogen peroxide production and chromosome aberration production with X-rays may also be cited (cf. THODAY and READ 1949; GILES 1952). The present results with fast neutrons may also be interpreted on the peroxide hypothesis if the effect of oxygen on hydrogen peroxide production by fast neutrons is intermediate between that with X-rays and alpha particles. There appear to be few quantitative experimental data on this point, but the results of ALLEN (1948) indicate that hydrogen peroxide does occur in oxygen-free water irradiated with neutrons.

It appears more probable, however, that hydrogen peroxide alone is not responsible for the entire radiation effect, particularly following X-irradiation, either in the presence or in the absence of oxygen. With this radiation it seems very likely that the hydroperoxyl radical (HO_2), which is produced by the reaction of dissolved oxygen with H atoms, plays a major role when oxygen is present, even though hydrogen peroxide molecules resulting from the subsequent reactions of these radicals with additional H atoms may also produce some of the biological effect. Thus, it may well be that reactions produced by OH and HO_2 radicals (and possibly by H atoms), rather than by hydrogen peroxide, are principally effective in producing chromosome aberrations and that a certain average concentration of such radicals may be required for a chromosome break to result. The existence of an inverse correlation between ionization density and the effect of oxygen could then be interpreted as resulting from competitive interactions, involving the primary radicals or atoms (OH and H) produced in irradiated water, and molecular oxygen. The radiochemical evidence (LEA 1947; GRAY 1949) indicates that the distribution of these primary products (OH and H) will be quite different for various radiations and will resemble in general the pattern of primary ionization distribution. Thus, following the passage of an alpha particle through water there will arise a high concentration of H atoms and OH radicals close to the axis of the particle path. The distribution of H atoms will presumably be somewhat more peripheral, but will still be relatively concentrated. Conversely, following X-irradiation, both OH radicals and H atoms will be more widely spaced (except near the end of the secondary electron path). These distributions following fast neutron irradiation would be intermediate. Under these circumstances, the high concentration of OH radicals produced by alpha radiation would favor efficient break production. Further, the relative proximity of H atoms would favor their recombination to form molecular hydrogen. Thus, even with oxygen present, there would be little opportunity for HO_2 radicals to be formed with alpha radiation, and consequently little or no effect of oxygen on aberration yield. Following X-irradiation a different situation would exist. The relative concentration of OH radicals would be less and the efficiency of break production consequently reduced. However, the more widely

spaced distribution of H atoms would make their recombination less likely, and with oxygen present would favor HO_2 production and a consequent increase in aberration yield. With fast neutrons, an intermediate distribution of radicals should arise, depending on the energies of the recoil protons, and some oxygen effect might be expected. In summary, on this interpretation, the presence of oxygen during irradiation would have little effect when radiations having a high specific ionization were used, since under these circumstances the reaction $H + H \rightarrow H_2$ would be favored over $H + O_2 \rightarrow HO_2$. With radiations having a low specific ionization, the opposite situation would obtain and a pronounced effect of oxygen would result.

It should be pointed out, however, that the possibility also exists that hydrogen peroxide molecules may be involved in alpha particle effects, even if they prove to be relatively unimportant in X-ray effects, since the close proximity of OH radicals along the paths of these particles should favor their rapid reaction to form hydrogen peroxide. At the present time it does not appear possible to decide whether OH radicals or hydrogen peroxide molecules are primarily responsible for the alpha-particle effects. Further, there is the additional possibility that active products of secondary reactions, such as organic peroxides which are known to be mutagenic (DICKEY *et al.* 1949), are involved. This seems rather unlikely, however, in view of the apparent localization of the effects to the immediate vicinity of particle paths, a point which will be discussed later.

Previous experiments (GILES and BEATTY 1950) on the relationship between the percentage of oxygen present during X-irradiation and aberration frequency demonstrated that there is still an appreciable aberration yield even when oxygen is removed as completely as possible from the cells before irradiation. Such residual aberrations could arise either from the indirect effects of OH radicals produced in oxygen-free water, as has been assumed in the preceding discussion, or from the direct effect of the radiation on the chromosomes. In an experimental attempt to distinguish between these alternatives, irradiations were performed in hydrogen under pressure in order to facilitate the removal of OH radicals by promoting the back reaction: $H_2 + OH \rightarrow H_2O + H$. This treatment did not reduce the aberration frequency significantly (GILES and BEATTY 1950). The failure to find a hydrogen effect may be interpreted as indicating that OH radicals are not involved in aberration production. However, since it has not been experimentally verified that the postulated back reaction actually can occur in the biological system under test, the negative evidence from this experiment is not conclusive. It still seems likely in view of the high water content of this system, that OH radicals are responsible (at least in part) for break production in the absence of oxygen.

It will be recalled that the relationship between interchange frequency (both chromosome and chromatid) and fast neutron dosage is linear whether the irradiation takes place in the presence or in the absence of oxygen. For X-rays, however, this relationship is nonlinear in both instances. These data provide further evidence for a relative localization of radiation effects, whether such

effects are produced directly or indirectly. Even though indirect action by way of radical formation may be involved, both breaks resulting in an interchange must be produced in most instances by radicals arising along a single particle path with neutrons, but along two separate particle paths with X-rays.

SUMMARY

An experiment has been performed to determine the effect of oxygen on the production by fast neutrons (derived from uranium fission in the Oak Ridge nuclear reactor) of chromosomal aberrations in Tradescantia microspores. Inflorescences were exposed in a special airtight lucite chamber in atmospheres of either pure oxygen, pure nitrogen, or pure helium. Studies were made of both chromatid aberrations (at 24 hours following treatment) and chromosome aberrations (at four and five days after treatment).

The results of this experiment indicate that, in the absence of oxygen, the frequencies of all types of aberrations (in both chromosome and chromatid categories) are reduced when equal dosages of fast neutrons are compared. However, a quantitative comparison of these results with previous observations on the oxygen effect with X-rays and with alpha particles in Tradescantia and Vicia indicates that this effect with neutrons is intermediate, being greater than that found with alpha particles and less than that found with X-rays. There is thus an inverse relationship between the specific ionization (ionization density) produced by a given type of radiation and the magnitude of the oxygen effect on chromosome aberration production. This relationship is interpreted in terms of differential spatial distributions, and consequent interactions, of the primary radiation products (OH radicals and H atoms), in the presence of oxygen, following the exposure of cells to various types of radiations.

The relationship between fast neutron dosage and aberration frequency is linear for all types of aberrations (including both chromatid and chromosome interchanges) in both the presence and absence of oxygen. This finding provides further evidence for a relative localization of radiation effects to the immediate vicinity of particle tracks within a cell whether such effects are considered to be produced indirectly or directly.

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A COMPARATIVE STUDY OF MUTATION RATES IN TWO ECOLOGICALLY DIVERSE SPECIES OF DROSOPHILA[1]

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THE process of mutation is the only known source of the genetic raw materials from which evolutionary changes can be built by natural selection and other agencies. It may well be doubted if either the direction or the tempo of evolutionary changes are often determined directly by the mutation rates. This much seems, nevertheless, certain: the presence of a store of genetic raw materials may be a limiting factor of evolution. The extreme scarcity of comparative data on the rates of spontaneous mutation in different organisms is therefore unfortunate. Quantitative data on mutation rates in homologous chromosomes of different species seem to be altogether absent. The present article reports the results of an exploratory attempt in this direction.

We deemed it probable that biologically most meaningful data might be obtained by comparison of species which, although systematically closely related, differ in their modes of life and in environmental adaptations. Two species have, therefore, been selected for the first attempt, namely, *Drosophila willistoni* and *Drosophila prosaltans*. Both species are members of the neotropical fauna, and both belong to the same subgenus, Sophophora. More important still, they have identical metaphase chromosome groups, and their chromosomes can be homologized on the basis of the mutants which occur in them (SPASSKY, ZIMMERING and DOBZHANSKY 1950; SPASSKY and DOBZHANSKY 1950). But they are quite different ecologically. *D. willistoni* is one of the commonest species of Drosophila in the American tropics, being the commonest species in many regions. *D. prosaltans* is a rare species in much of its gross distribution area. *D. willistoni* is ecologically versatile and occurs in a great variety of habitats. *D. prosaltans* is evidently ecologically specialized, since it occurs very spottily and then only in limited numbers (DOBZHANSKY and PAVAN 1950).

One of the chromosomes of *D. willistoni* and *D. prosaltans*, namely, the second chromosome, contains mostly the same loci which are carried also in the second chromosome of *D. melanogaster* (SPASSKY *et al.* 1950). This makes our data comparable with the relatively very abundant data recorded in the genetical literature which deals with the last named species. Although *D. willistoni* and *D. prosaltans* are sometimes found in orchards and gardens, they are wild species, not intimately associated with man. *D. melanogaster* is, in the Western Hemisphere, a commensal dependent on man-made environ-

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ments. The third chromosomes of *D. willistoni* and *D. prosaltans* carry loci borne in the second chromosomes of *D. pseudoobscura* and *D. persimilis*. The latter are species living in the temperate zones of western North America. The mutation rates in their second chromosomes are under study.

THE EXPERIMENTAL PROCEDURE

Our purpose has been to determine the rates of spontaneous mutations in the autosomes of *D. willistoni* and *D. prosaltans*. Recessive lethals and extreme semilethals have been chosen for study, since the detection of this class of mutations is at the same time technically simple and independent of the individual ability to notice structural alterations in the fly's body. This "personal equation" might make the data on visible mutants of questionable validity. But spontaneous mutations which produce recessive lethals are relatively rare events. Their rarity has enforced the use of a technique of accumulation of mutations in chromosomes transmitted from generation to generation in flies which carried also some convenient marking genes derived from certain laboratory strains. We realize that the use of this technique entails some risks, pointed out especially by IVES (1950). The mutation rates may undergo changes in strains long kept in the laboratory. To mitigate this source of error, we have used marking genes outcrossed to recently collected wild strains. The methods used are variants of that described for *D. pseudoobscura* by DOBZHANSKY and WRIGHT (1941).

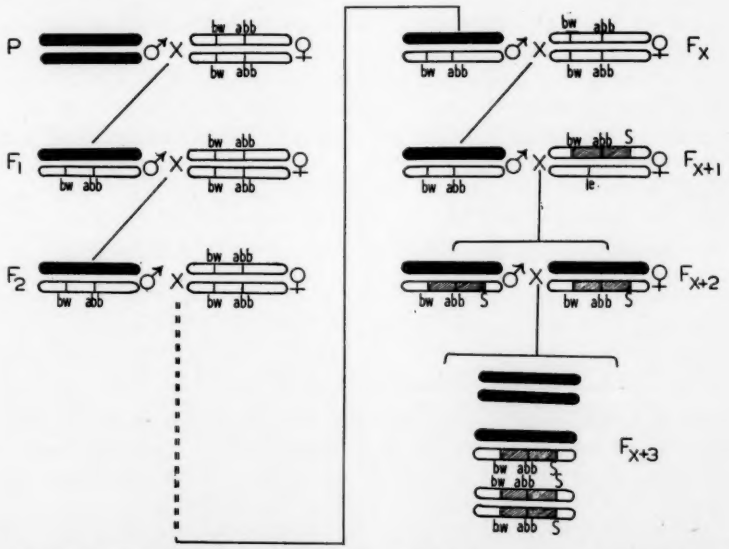

FIGURE 1.—The technique used for "accumulation" of recessive mutant genes in the second chromosome of *Drosophila willistoni*. Black chromosomes in which the mutants are accumulated; white chromosomes with marker genes; cross-hatched, inversions.

The second chromosome of D. willistoni. Twenty-two wild strains were chosen, the second chromosomes of which were tested by PAVAN *et al.* (1951) and were found to be free of mutant genes reducing the viability of the homozygotes. Six of these strains were derived from wild progenitors collected at Belem, State of Pará, six collected at Mogi, State of São Paulo, and ten at Pirassununga, State of São Paulo, all in Brazil. A total of 429 males from these strains were outcrossed singly to females homozygous for the second chromosome recessives abbreviated and brown (*abb bw*), as shown in figure 1.

TABLE 1

Mutations in the second chromosome of Drosophila willistoni.

Origin	Number of strains	Chromosome-generations	Lethals	Semilethals	Visibles
Mogi 44	13	273	4	....	....
" 43	15	315	8	1	1
" 17	13	273	2	1	....
" 16	14	294	3	....	....
" 39	15	315	4	....	....
" 4	16	336	3	....	1
Mogi total	86	1806	24	2	2
Pirassununga 4	19	228	....	1	....
" 47	14	168	2	....	....
" 39	18	216	1	....	....
" 21	20	240	2	1	....
" 23	16	192	....	1	....
" 1	17	221	2	....	1
" 35	19	247	2	1	....
" 48	18	234	1	....	....
" 52	18	234	....	1	1
" 55	17	221	....	1	....
Pirassununga total	176	2201	10	6	2
Belem 9	30	360	....	2	....
" 58	28	336	1	....	....
" 63	27	324	2	1	....
" 67	30	360	2	....	....
" 56	27	324	1	1	....
" 59	25	300	....	1	1
Belem total	167	2004	6	5	1
Grand total	429	6011	40	13	5

In the next and in the following generation, 6 to 10 males, wild-type in phenotype but heterozygous for *abb bw*, were taken from each of the 429 lines and outcrossed again to homozygous *abb bw* females. Such outcrosses were repeated for 13 to 21 generations in various lines (table 1).

In every outcross generation the wild second chromosomes of the males are exposed to the risk of acquiring recessive mutant genes. The total number of such exposures, chromosome-generations, in an experiment is equal to the product of the number of the lines multiplied by the number of generations

during which these lines were outcrossed (table 1). The recessive mutants cannot however manifest themselves, because they are transmitted from generation to generation in heterozygous condition. To detect the mutants, a single wild *abb bw* male from each line is crossed to females of the " analyzer stock." This stock carries in one of the second chromosomes the dominant gene Star (*S*), the recessives *abb* and *bw*, and an inversion which suppresses practically all recombination in the heterozygotes. The opposite second chromosome has a " balancing " recessive lethal (*le*, figure 1).

In the next generation, virgin females and males which show *S* but not *abb* or *bw* are selected and inbred. If no mutation is contained in the experimental second chromosome (black in figure 1), the progeny will consist of *S* and wild-type flies in a ratio approaching 2:1. A recessive lethal mutation will manifest itself by the absence of the wild-type flies in a given culture. A semilethal permits some wild-type flies to appear but with frequencies below 5:1. Mutants which produce visible external changes or appreciable retardations of the development can also be detected (cf. PAVAN *et al.* 1951).

The third chromosome of D. willistoni. Although different mutant markers and different analyzer stocks were used, the experimental procedures were in all instances analogous to that described above for the second chromosome. Nineteen strains were chosen which were known to be free of recessive mutants in the third chromosomes. The progenitors of ten of these strains came from Pirassununga, State of São Paulo, and of nine strains from Belem, State of Pará, Brazil (table 2). Males from these strains were crossed to females homozygous for the third chromosome recessive pink (*p*), and the heterozygous wild/*p* sons were outcrossed for 12 to 21 generations to homozygous *p* females. A total of 323 lines were established in which these outcrosses were made, and, consequently, the accumulation of recessive mutants in the wild third chromosomes was going on. The number of the chromosome-generations tested was 5715 (table 2).

The analyzer stock carried in the third chromosome the dominant mutant Delta, the recessive pink, and an inversion suppressing the recombination, and a " balancing " lethal in the opposite chromosome. Males wild/pink were crossed to females of the analyzer stock, and in the progeny Delta non-pink females and males were selected and inbred. The progeny consisted of Delta and wild-type flies in a ratio approaching 2:1, provided that no mutants were carried in the chromosome analyzed. Recessive lethals in that chromosome manifested themselves by producing cultures with Delta and no wild-type flies. Recessive semilethals allowed a few wild-type individuals to survive.

The second chromosome of D. prosaltans. We had at our disposal strains of this species only from Belem, State of Pará. Eight such strains, which were free of mutant genes in the second chromosome, were outcrossed to a strain homozygous for the recessive plexus (*px*). In 156 parallel lines, the wild/plexus males were outcrossed for 14–21 generations to plexus females. Thus, a total of 5098 chromosome-generations were exposed to the chance of acquisition of recessive mutations (table 4). To reveal the presence of these

mutants, the wild/*px* males were outcrossed to a strain of the composition *L px/px* (Lobe being a dominant gene). A single Lobe male was selected in the progeny of each line and outcrossed to females of a strain which carried in one of the second chromosomes the dominants Curly (*Cy*), Star (*S*), and Plum (*Pm*), and an inversion which suppresses the recombination in the second chromosome. The genetic constitution of the Lobe males was, of course, wild/*L px*. In the offspring of the cross wild/*L px* male × *Cy S Pm Inv*/Lethal females and males showing *Cy*, *S*, and *Pm*, but not *L*, were selected and inbred. A segregation in the ratio approaching 2 *Cy S Pm*: 1 wild is expected in the progeny. If the wild chromosome carries a recessive lethal, the wild-type fails to appear. The analysis process just outlined requires one generation more than in the cases described above. This is due to the absence in the *Cy S Pm Inv* chromosome of a convenient recessive gene, necessitating the interposition of the intermediate step involving *L px*.

The third chromosome of D. prosaltans. Nine strains derived from flies collected at Belem and known to be free of third-chromosome recessive mutants were outcrossed to females homozygous for the recessive abdomen rotatum (*ar*). For 16 to 18 generations, wild/*ar* males were then outcrossed to *ar/ar* females in 391 parallel strains (table 5). The number of chromosome-generations obtained was 6547. To reveal the recessive mutations that have accumulated in the wild third chromosomes, single males from each of the 391 strains were outcrossed to females of the composition Δ *ar Inv*/lethal. The gene Δ, Delta, is a dominant; the inversion suppresses the recombination in the third chromosome. In the next generation, females and males showing Delta, but not *ar*, were selected and inbred. The progeny is expected to consist of Delta and wild-type flies in a ratio about 2: 1. Third chromosome recessive lethals produce cultures consisting of Delta flies only.

The experiments with the accumulation of lethals in the chromosomes of *D. willistoni* and *D. prosaltans* continued for more than two years. Since we do not have facilities for keeping hundreds of cultures for this length of time at constant temperature, the cultures were kept most of the time in a laboratory working room in which the temperature fluctuated, with few exceptions, between 22° and 25°. During the hot period of the summer, the cultures were, however, kept in a constant temperature room at 25°. The average temperature may be taken to be close to 24°C.

In the final generation (X + 3 in figure 1) no complete counts of the flies were made in most cultures. The cultures were etherized when the hatching of the flies was mostly completed, and the flies were inspected on a white plate under the dissecting microscope. The presence of a large number of wild-type individuals led to the line being scored as " normal." If the wild-type class was absent or rare, the culture was kept until it was exhausted. Most of the semi-lethals are so close to complete lethality that their scoring causes no difficulty. One culture contained, however, no wild-type flies when first examined and was accordingly presumed to contain a lethal. A later count disclosed a large number of wild-types; here a recessive mutation which slowed down the development of the homozygotes was evidently involved.

MUTATIONS IN THE SECOND CHROMOSOME OF D. WILLISTONI

A summary of the data is shown in table 1. Altogether 40 complete lethals, 13 semi-lethals, and 5 visible mutants were observed. Of the visibles, 2 were also semi-lethal.

Our technique of the detection of the lethals permits the finding of the second chromosomes which are lethal, or semi-lethal, to the homozygotes. It does not permit discrimination between chromosomes which contained only a single lethal, or two or more lethals or semi-lethals. A correction factor must be introduced before the mutation rates can be estimated. It is assumed that the likelihood of a lethal, or semi-lethal, mutation taking place in a chromosome is not affected by the presence in that chromosome of other lethal mutations. The possibility that two semi-lethal mutations can make a chromosome completely lethal is neglected. Let, then, the number of strains tested be X, the number containing lethals or semi-lethals be Y, and the number free of lethals and semi-lethals be Z. The Poisson formula gives, then,

$$Z/X = e^{-m},$$

where e is the base of natural logarithms, and m the mean number of lethals and semi-lethals per strain. This can also be written in the form:

$$m = \ln X - \ln Z$$

The corrected numbers of the lethal and semi-lethal mutations observed in the second chromosome are shown in table 3, together with their frequencies in percent of the chromosome-generations in which the mutations had had chance to occur. The 95 percent confidence limits of the estimated mutation rates are also given. These limits have been calculated from the formula:

$$p = [2nf + 4 \pm \sqrt{(2nf+4)^2 - 4nf^2(n+4)}]/(2n+8)$$

In this formula, p and f are the true frequency and the observed frequency respectively of the lethals and semi-lethals, and n the number of chromosome-generations carried out.

It can be seen that, on the average, one in a hundred second chromosomes of *D. willistoni* acquires a new lethal or semi-lethal mutation in every generation (the figure actually observed is 0.983 percent). It must, however, be emphasized that significant variations in the mutation rates have been observed within the species. Namely, the chromosomes of Mogi origin are significantly more mutable than those from Pirassununga or from Belem. No significant heterogeneity has appeared in these experiments between the different strains from the same locality (tables 1 and 3).

Since 5 recessive mutants with visible external effects were observed in 6011 chromosome-generations (table 1), the frequency of such mutations appears to be about 0.1 percent. It should, however, be kept in mind that some of the visibles were also semi-lethal (see above).

MUTATIONS IN THE THIRD CHROMOSOME OF D. WILLISTONI

The data are summarized in tables 2 and 3. Among the 323 strains tested, 35 carried third chromosomes which were completely lethal when homozy-

TABLE 2

Mutations in the third chromosome of Drosophila willistoni.

Origin	Number of strains	Chromosome-generations	Lethals	Semilethals	Visibles
Pirassununga 48	14	168	1	....	1
" 44	17	204	1	....	....
" 67	15	180	1	1	....
" 41	17	204	2	....	....
" 149	19	228	1	1	....
" 56	11	198	....	....	1
" 60	18	324	1	2	1
" 68	18	324	2	2	....
" 72	15	270	2	....	....
" 74	16	288	3	....	1
Pirassununga total	160	2388	14	6	4
Belem 133	12	252	1	1	....
" 114	15	315	2	1	1
" 121	14	294	1	....	....
" 113	14	294	4	....	....
" 130	12	252	3	1	....
" 50	23	460	5	2	2
" 58	25	500	2	....	....
" 17	25	500	2	1	1
" 59	23	460	1	....	....
Belem total	163	3327	21	6	4
Grand total	323	5715	35	12	8

gous, 12 carried semi-lethals, and 8 carried visibles (4 of which were also semi-lethal). When corrected, these data yield the frequency 0.894 percent of lethals and semi-lethals per generation.

Since the acrocentric third chromosome is about three-fifths as long as the metacentric second, the former is expected to show a lower mutation rate than the latter. The difference observed (0.89 and 0.98 percent per generation, table 3) is not statistically significant, although it is in the right direction. Un-

TABLE 3

Corrected frequencies of lethal and semilethal mutations in Drosophila willistoni.

Origin	Chromosome-generations	Mutations observed	Frequency (in percent)	Confidence limits
Second chromosomes				
Mogi	1806	30.96	1.714	1.203–2.438
Pirassununga	2201	16.77	0.762	0.472–1.231
Belem	2004	11.38	0.568	0.317–1.016
Total	6011	59.11	0.983	0.760–1.272
Third chromosomes				
Pirassununga	2388	21.45	0.898	0.585–1.338
Belem	3327	29.67	0.892	0.624–1.294
Total	5715	51.12	0.894	0.678–1.179

fortunately, we have not tested third chromosomes from the population of Mogi, which showed high mutation rates for the second chromosome. If second and third chromosomes from Pirassununga and from Belem are compared to each other, the mutation rates are ostensibly even higher in the third than in the second chromosomes (table 3). The confidence limits are, however, so wide that the data on hand do not contradict the assumption that the true mutation rates are actually higher in the second chromosomes.

MUTATIONS IN THE SECOND CHROMOSOME OF D. PROSALTANS

A total of 19 lethal, 5 semi-lethal, and 6 visible mutations (3 of them also semi-lethal) were observed among 5098 chromosome-generations (tables 4 and 6). This gives a corrected frequency of 0.498 percent per chromosome-generation. This value is only half as large as that observed for the mutation rate in the comparable chromosome of *D. willistoni,* and the difference is statistically quite significant (compare tables 3 and 6). The situation is complicated, however, by the fact that the high average mutation rate among the

TABLE 4

Mutations in the second chromosome of Drosophila prosaltans.

Origin	Number of strains	Chromosome-generations	Lethals	Semilethals	Visibles
Belem 1	34	714	3	....	....
" 2	56	784	6	3	3
" 3	57	1197	5	1	1
" 8	43	903	3	....	1
" 9	21	420	1	....	1
" 10	15	300	....	1	....
" 30	15	300	....	....	....
" 40	24	480	1	....	....
Total	265	5098	19	5	6

D. willistoni second chromosomes is due to the inclusion of the strains of Mogi origin. If one compares the mutation rates in the second chromosomes in strains of both species derived from the Belem population, the figure obtained for *D. willistoni* is somewhat higher than that for *D. prosaltans,* but the difference is no longer significant statistically. It must furthermore be noted that the frequency of mutations apparently varies even among the different strains of *D. prosaltans,* even though these are all derived from flies collected at Belem. Thus, the strains Belem-2 and Belem-3 appear to be more mutable than the rest, although this heterogeneity is not quite significant.

MUTATIONS IN THE THIRD CHROMOSOME OF D. PROSALTANS

Summaries of the data are given in tables 5 and 6. One of the strains, Belem-5, is more mutable than the others. It has produced 15 lethals and semi-lethals in 608 chromosome-generations, while the remaining eight strains gave 22 lethals and semi-lethals among 5939 chromosome-generations.

The combined data give a mutation rate 0.638 percent, which is signifi-

TABLE 5

Mutations in the third chromosome of Drosophila prosaltans.

Origin	Number of strains	Chromosome-generations	Lethals	Semilethals	Visibles
Belem 2	53	901	2	1	1
" 7	58	986	2	....	1
" 11	48	864	2	....	....
" 6	40	680	5	2	....
" 3	37	592	4	1	....
" 5	38	608	14	1	....
" 4	44	748	1	....	....
" 1	36	576	1	1	....
" 6	37	592	....	....	....
Total	391	6547	31	6	2

cantly higher than the observed rate for the second chromosome. As stated above, the third chromosome is expected to produce fewer mutations than the second. It is probable that the inclusion of the Belem-5 strain has made the estimate too high. The more interesting, then, is the comparison of the third chromosome of *D. willistoni* and *D. prosaltans*. The 95 percent confidence limits for the former species are 0.678–1.179, and for the latter, 0.467–0.869. Although these confidence limits overlap, the observed values for either species are outside the confidence limits for the other (compare tables 3 and 6).

Despite the complications encountered, it looks as though *D. willistoni* as a species is characterized by higher mutation rates than *D. prosaltans*. It should be noted in that connection that the life cycle in the former species is shorter than it is in the latter—approximately 12 and 16 days respectively at room temperature. If computed per unit of physical time, the difference in the mutation rates would, then, appear the more significant.

COMPARISON WITH D. MELANOGASTER

The second chromosome of *D. melanogaster* contains mostly the same gene loci which are carried also in the second chromosomes of *D. willistoni* and *D. prosaltans*. Comparisons of the mutation rates in the second chromosomes of these species are, therefore, legitimate. This is not the case with the third chromosomes. The third chromosomes of *D. willistoni* and *D. prosaltans* correspond to only a part of the third chromosome of *D. melanogaster* (SPASSKY, ZIMMERING and DOBZHANSKY 1950; SPASSKY and DOBZHANSKY 1950).

By far the most extensive data on mutation rates in the second chromosome

TABLE 6

Corrected frequencies of lethal and semilethal mutations in Drosophila prosaltans.

Chromosome	Chromosome-generations	Mutations observed	Frequency (in percent)	Confidence limits
Second	5098	25.37	0.498	0.338–0.740
Third	6547	41.80	0.638	0.467–0.869

of *D. melanogaster* are those of MULLER (1928). He found 69 lethals (and presumably extreme semi-lethals) in 17,572 chromosome-generations, or a frequency of 0.40 ± 0.01 percent. DUBININ (1946) has summarized the data obtained by himself, by BERG, OLENOV, and their collaborators. In most strains DUBININ and BERG found mutation rates ranging from 0.33 ± 0.07 to 1.27 ± 0.42 percent, with a mean close to 0.5 percent per generation. Some strains were, however, appreciably more mutable. OLENOV obtained generally much higher rates (up to 5.9 percent); the validity of his data may be questioned. IVES (1945, 1950), in cultures carried at 22 to 28°C, obtained mutation rates of 0.51 ± 0.13, 0.93 ± 0.18, 1.45 ± 0.28, and 0.87 ± 0.26 in different experiments. He found also a strain with a very high mutation rate (up to 7.16 percent). Excluding this strain, IVES obtained 81 lethals in 9034 chromosome-generations, or 0.89 ± 0.10 percent. WALLACE (1951) and WALLACE and KING (1951) obtained mutation rates between 0.4 and 0.5 percent.

Because of the variations in the mutation rates observed among strains of the same species, comparison of different species is obviously an hazardous procedure. Nevertheless, it would seem that mutation rates in the vicinity of 0.5 percent may be regarded reasonably close to typical for the second chromosomes of *D. melanogaster* at room temperature. This coincides with the figure obtained for *D. prosaltans* (table 6), while in *D. willistoni* only the Belem strains gave a similarly low figure, while the average mutation rate in our material is almost twice as high (table 3).

MUTATION RATES PER LOCUS

The estimates of the mutation rates arrived at above refer to whole chromosomes. Since a chromosome contains many gene loci capable of giving rise to recessive lethal alleles, the different mutants are mostly non-allelic, and the flies which carry lethals in both chromosomes of a pair are viable. The frequency of allelism among lethals is evidently a function of the number of loci in a given chromosome which produce lethals by mutation. If the assumption is made that the mutation rates of all the loci are uniform, then the number of these loci can be estimated from the known frequency of allelism in a random sample of the lethals. Such estimates have been made for the third chromosome of *D. pseudoobscura* (DOBZHANSKY and WRIGHT 1941) and for the second chromosome of *D. melanogaster* (IVES 1945; WALLACE 1950). Since the second chromosome of *D. melanogaster, D. willistoni,* and *D. prosaltans* contain mostly the same loci, the estimates of IVES and WALLACE can be considered valid for any one of the three species. The estimate of IVES is 495 loci (285 and 705 being the 95 percent confidence limits), and that of WALLACE is 400 loci (the limits 234–718). Still assuming that all the loci are equally mutable, this would mean that the average mutation rate per locus in the second chromosome of *D. willistoni* is of the order of 2.2×10^{-5} (the limits being 1.3×10^{-5} and 4.2×10^{-5}). The corresponding estimates for *D. melanogaster* and *D. prosaltans* would be half as large (of the order of 1.1×10^{-5}).

If this assumption of uniform mutability of all loci is incorrect, as it almost

certainly is, then the number of the loci in the second chromosome is underestimated, and the average mutability per locus is overestimated. The upper limit of the number of the loci in the chromosome can be surmised on the basis of the known numbers of discs visible in that chromosome in the salivary gland cells. For the second chromosome of *D. melanogaster* this number is, according to BRIDGES (1942) about 1940 (803 in the left limb, and 1136 in the right limb). The number of the genes which can mutate to lethal alleles can hardly be greater than the number of the discs, and it is probably smaller (since some of the genes may not be completely essential to life). With 1940 loci, the average mutation rate per locus in the second chromosome of *D. willistoni* would be of the order of 5×10^{-6}, and in *D. melanogaster* and *D. prosaltans* about half as large. We believe the estimates based on the rates of allelism of the lethals to be closer to the truth than those based on the numbers of discs in the salivary gland chromosomes.

No data on the frequencies of allelism for third-chromosome lethals in *D. willistoni, D. prosaltans,* and *D. melanogaster* are available. The third chromosomes of the first and the second species are presumed to contain the loci

TABLE 7

Estimated average mutation rates per lethal-producing locus in different species (these estimates are more likely to be overestimates than underestimates—see text).

Species	Second chromosome	Third chromosome
D. melanogaster	1.1×10^{-5}	....
D. pseudoobscura	....	1.1×10^{-5}
D. willistoni	2.2×10^{-5}	3.0×10^{-5}
D. prosaltans	1.1×10^{-5}	2.1×10^{-5}

which in *D. melanogaster* lie in the right limb of the third chromosome. BRIDGES (1942) counted 1178 discs in that limb in the salivary gland cells. Taking this to be the number of the mutable loci, the average mutation rate per locus in *D. willistoni* would be 8×10^{-6}, and in *D. prosaltans* 5.4×10^{-6}. Those are minimal estimates, comparable to the 5×10^{-6} value for the second chromosome of *D. willistoni* (see above).

It may also be pointed out that DOBZHANSKY and WRIGHT (1941) estimated the minimum number of lethal-producing loci in the third chromosome of *D. pseudoobscura* to be about 287 (on the basis of the rate of allelism). Since the mutation rate in the chromosome is 0.31 ± 0.03 percent per generation, the mutation rate per locus will be at most 1.1×10^{-5}. This chromosome is a homologue of the right limb of the second chromosome of *D. melanogaster,* in which BRIDGES (1942) counted 1136 discs. The minimum number of loci in the third chromosome of *D. willistoni* and *D. prosaltans* may, then, be close to $287 \times 1136/1178$, or about 300 loci. On this basis, the mutation rate per third chromosome locus in *D. willistoni* should be about 3×10^{-5}, and in *D. prosaltans,* about 2×10^{-5}. The estimates of the mutation rates, based on the observed frequencies of allelism, are summarized in table 7.

DISCUSSION

It is very often observed that populations of some wild or domesticated animal or plant species contain a greater amount of genetic variability than do populations of other species. Some systematists, and even some geneticists, believed that the greater variability was due to higher mutation rates. This is, of course, by no means always the case. A more mutable species may well be the least variable one, if natural selection eliminates mutations soon after they arise. Conversely, adaptive polymorphism can be maintained without mutation. Comparative studies on mutation rates in different but related species have actually been exceedingly few. STADLER (1929) found that the frequencies of mutations per unit of X-ray treatment are greater in diploid species of wheats and oats than in tetraploid species, and in the tetraploids greater than in the hexaploids. This is almost certainly a consequence of sheltering of the mutants by the duplicate normal alleles carried in the semi-homologous chromosome sets in the polyploids. TIMOFEEFF-RESSOVSKY (1936) made a qualitative comparison of mutants in *D. funebris* and *D. melanogaster*. The former species produces relatively more semi-dominant mutants, more mutants with weak penetrance, mutants affecting the wing venation, and fewer color changes than the latter species. The absolute frequencies of the mutations were not determined.

Very interesting studies on mutation rates in different geographic populations of *D. melanogaster* have been published in a series of brief communications by BERG (1941–1942). She has claimed that the mutability varies greatly from population to population, and that the variations can be correlated with the ecological status of the populations in question. BERG regards the mutation rate as a fairly plastic trait which can undergo rapid adaptive changes in the process of evolution. The presence of an abundant store of genetic raw materials from which adaptive changes can be constructed makes any Mendelian population—from a species to a local race—malleable in the evolutionary sense. On the other hand, since most mutations are harmful, high mutation rates reduce the immediate fitness of some individuals, and indirectly of populations. Low mutation rates favor the immediate fitness at the expense of evolutionary plasticity. Natural selection will, then, strike a compromise. Plasticity is most important when a species or a race is broken up into numerous competing colonies. In undivided populations the competition occurs chiefly among individuals, rather than among groups. Immediate fitness takes the precedence in undivided populations, especially in small and isolated ones which live in relatively uniform environments. These ideas have been elaborated further and generalized in the important book of SCHMALHAUSEN (1949).

The experimental evidence adduced by BERG (*l.c.*) in favor of her ideas is, unfortunately, very meager. As pointed out by DUBININ (1948) and others, her findings require confirmation. We believe that comparison of mutation rates in species of ecologically different types is to be preferred to comparisons of populations of the same species, as a method of testing the validity of the

BERG-SCHMALHAUSEN views. If mutation rates are adaptively modified by ecological conditions, the divergence among species may be expected to be greater than among populations of a single species. This is important because comparative studies on mutation rates in higher organisms are necessarily laborious, and microorganisms present, from the evolutionary standpoint, situations *sui generis*. Even in Drosophila, the experimental errors of most determinations of the mutation rates are considerable. The estimation of the number of gene loci in the chromosomes which produce recessive lethals by mutation is even less reliable. The estimates of the mutation rates per locus are, thus, subject to compounding of errors. Nevertheless, the figures obtained by us for *D. willistoni* seem to be consistently higher than those for the other three species—*D. melanogaster, D. pseudoobscura,* and *D. prosaltans*. The average mutation rate per locus is between 2.2 and 3.0 mutations per 100,000 chromosome-generations in *D. willistoni*. In the other species the estimates are between 1.1 and 2.1 per 100,000 (cf. table 7).

It is especially suggestive that *D. prosaltans* shows lower mutation rates than *D. willistoni*. The experiments concerning these species were carried in part simultaneously and by the same group of investigators. The two species are sympatric over most of their known geographic ranges, and thus adapted to more or less similar climatic conditions. But, as stated in the introduction, they differ sharply in that *D. willistoni* is very common and ecologically versatile, while *D. prosaltans* is rare, occurs in small isolated colonies, and apparently exploits some specialized ecological niches. The observed difference in the mutation rates is, thus, in accord with expectation based on the BERG-SCHMALHAUSEN hypothesis.

We admit that our data must also be regarded as preliminary, and that further comparative studies on the mutation rates in different species are needed. We have encountered variations in the mutation rates within the species which we studied. The objection may be raised that this vitiates the conclusion that the species as such are differently mutable. Indeed, the mutation rate in the second chromosome of the Belem population of *D. willistoni* seems not significantly higher than that in the second chromosomes of the population of *D. prosaltans* from the same region (cf. tables 3 and 6). To be sure, the mutation rates in the third chromosomes are higher in the Belem population of *D. willistoni* than in *D. prosaltans*. It should, however, be noted that if the Belem population of *D. willistoni* were to exhibit a lower mutability than those of Mogi and Pirassununga (table 3) this would also agree with the BERG-SCHMALHAUSEN hypothesis. As shown by DACUNHA, BURLA and DOBZHANSKY (1951) the Belem population of *D. willistoni* contains much less inversion heterozygosis than do the populations of many other regions of Brazil. This seems to be connected with the fact that at Belem *D. willistoni* is not the ecologically dominant species which it is elsewhere, being displaced from this position by a related form, *D. paulistorum*. The Belem population of *D. willistoni* is thus ecologically limited to relatively few niches and approaches, in this respect, *D. prosaltans*.

SUMMARY

The rates of spontaneous mutations giving rise to recessive lethals and semilethals have been studied in the second and the third chromosomes of *Drosophila willistoni* and *Drosophila prosaltans*. The techniques used permitted "accumulation" of the recessive mutants in a series of generations in chromosomes transmitted from father to son.

The mutation rates varied significantly in different strains of the same species. Nevertheless, it seems that *D. willistoni* is, as a species, characterized by higher mutation rates than *D. prosaltans*. Estimates of the mutation rates per mutable locus in the two species just named, and also in *D. melanogaster* and *D. pseudoobscura*, are presented in table 7. It is regarded as probable that the greater mutability of *D. willistoni* may be correlated with its greater ecological versatility and with the occupation of a greater variety of ecological niches in the tropical habitats.

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THE INDUCTION OF TRANSLOCATIONS AND RECESSIVE LETHALS IN DROSOPHILA UNDER VARIOUS OXYGEN CONCENTRATIONS[1,2]

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A GREAT deal of attention has been paid, within the last few years, to the effect of exposing organisms to ionizing radiations while they were maintained in an environment of low oxygen concentration. In general, it was found that, irrespective of the biological effect studied or the organism used, the damaging effect of the radiation was reduced when the irradiation was performed in low oxygen tensions.

These results suggest that either or both of two chains of events (they need not be mutually exclusive) are taking place. The majority of oxygen effects are capable of explanation on the assumption that the primary event caused by radiation is not the direct absorption of the radiant energy within a particular macromolecular unit, but rather the energy is absorbed within relatively simple protoplasmic constituents (e.g., water) with the resulting formation of certain active products. It is these products which act on the macromolecular units to produce the observed effect and, in turn, the amount of these products formed is determined by the oxygen tension. On the other hand, a good deal of the oxygen effect data is compatible with the view that the number or kind of macromolecular units damaged by the radiation is not disturbed by low oxygen levels but rather the damage is more likely to be restored under these conditions.

The studies in Drosophila reported here were undertaken with two objectives in mind. In the first place, it was felt desirable to determine if the induction by X-rays of translocations in Drosophila follows the same type of relationship with various oxygen concentrations as does the induction of two-break aberrations in Tradescantia. Secondly, it was hoped that, by a comparison of data relating oxygen concentration to production of translocations with that relating oxygen tension to the induction of sex-linked recessive lethals, a decision could be reached as to whether the oxygen tension was affecting the number of primary events—chromosome breaks or point mutations—produced

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by the radiation, or whether it was merely altering the amount of rejoining of the broken chromosome ends.

MATERIALS AND METHODS

Translocation production was studied in *Drosophila virilis,* a species ideally suited for the detection of translocations by genetic methods because of the presence of five telocentric chromosomes of approximately equal length. The radiation was administered to 75 eight- or nine-day-old wild type males of the Pasadena strain. After treatment these males were mated to approximately 100 females containing recessive genetic markers on the four major autosomes: *b* 2–188.0, *t* 3–57.5, *cd* 4–32.0, *pe* 5–203.0. The males were removed from the culture bottle on the sixth day after treatment and the number of them still living was recorded. Either all the F_1 males or a randomly selected sample of them were mated individually in shell vials to females of the *b;t;cd;pe* stock—each vial being a testcross of a single irradiated sperm. The F_2 generation was examined phenotypically to determine if any translocations had been induced in the sperm of the treated male between any of the four autosomes or the Y chromosome. The number of F_2 individuals in each of the phenotypic classes was recorded for every translocation.

Drosophila melanogaster was used in studying the induction of sex-linked recessive lethals. Seventy-five males of the Oregon R strain, which had emerged three days previously, were irradiated and mated in a culture bottle to approximately 100 Muller-5 (sc^{81} *B In-S* w^a sc^8) females. On the sixth day after treatment the males were removed from the culture and the number living was recorded. As the F_1 offspring emerged, they were placed in aging bottles in order to increase the opportunity for mating between the F_1 heterozygous Bar females and their Muller-5 brothers. After all the offspring had emerged, a random sample of the F_1 females was taken and each female in the sample was placed in a shell vial either with one of her brothers or with a Muller-5 male from stock. An examination of the F_2 offspring in each vial indicates whether there were induced any recessive lethals on the X chromosome of a single irradiated sperm.

Since the lethality of some recessive lethals is a function of the environment, the decision to classify an X chromosome as one with a lethal is somewhat arbitrary. Therefore the following criteria were established: (1) if one or more wild type males were observed among the F_2 offspring, the irradiated X chromosome was classified as one not bearing any lethals. (2) In F_2 vials which did not contain any wild type males and in which eight or fewer Muller-5 males were present, an F_3 generation was produced by mating the F_2 heterozygous Bar females to Muller-5 males. These cases were classified as lethals if less than 5 percent of the F_3 males were wild type. (3) About 2 percent of the F_2 cultures which contained no wild type males had relatively few offspring. These were classified as lethals if there were more than seven Muller-5 males in either the F_2 or F_3 generation. If the number of Muller-5 males was less than this figure, they were classified as failures.

Since the criteria used in classifying an X chromosome as one containing a lethal are arbitrary, it is important that the environmental conditions remain as constant as possible. During the course of the investigation the flies were kept in a constant-temperature room maintained at 23 ± 1°C with a relative humidity of 50 ± 10 percent. The F_2 offspring were allowed to develop in 95 x 25 mm shell vials on a yeast-agar-sucrose medium slightly modified from that reported by CARPENTER (1950).

The flies were anesthetized and placed in a small cylindrical gauze capsule which was inserted in the irradiation chamber. The irradiation chamber consisted of a rectangular box (3⅜ × 1 × 1 in.) made of one-fourth-inch lucite with a stopcock fastened to each end. The lid of the chamber was secured by means of screws which drew the lid tight against a rubber gasket around the top of the chamber. The chamber containing the flies was then evacuated with a Cenco-Hyvac vacuum pump and a check was made for any leakage in the system by using a mercury manometer. The desired gas mixture was next flushed into the chamber until atmospheric pressure was reached. The evacuation and flushing of the gas was repeated five times in order to insure that the gas composition in the chamber was the desired gas mixture. During the fifth passage of gas through the irradiation chamber, it was closed off by means of the stopcocks and partially submerged in a water bath beneath the X-ray tube. Irradiation was not begun until 10 minutes after the chamber was placed in the bath which was maintained near 0°C by use of ice. Subsequent measurements of the temperature within the chamber using a series of copper-constantan thermocouples indicate that at the end of 10 minutes in the bath the temperature was approximately 8°C, and at 17 minutes had reached an equilibrium at 5°C. Immediately after the irradiation was completed the males were removed from the chamber and mated to the appropriate females.

The gases used were commercially prepared pure oxygen or nitrogen or mixtures of oxygen and nitrogen. Cylinders containing the following gases were used in these studies: pure nitrogen, 2 percent oxygen (tested 2.2 percent), 5 percent oxygen (tested 4.9 percent), 9 percent oxygen (tested 8.9 percent), 15 percent oxygen (tested 14.7 percent), compressed air, 21 percent oxygen (tested 20.9 percent), and pure oxygen. The nitrogen from the cylinder was passed over hot copper screening to remove any trace of oxygen which might be present.

The X-rays were administered the flies by means of a Victor machine with a mechanical rectifier and a universal type Coolidge tube. The machine was operated at 100 kvp and 5 ma with a ⅓-mm Al filter. Dosage measurements, made frequently during the course of the investigation, were taken with a Victoreen 100-r thimble chamber inserted within a dummy irradiation chamber which was placed in the water bath at the same location that the flies were treated. At the target distance used (24 cm), the dosage rate varied from 100 r/minute to 120 r/minute over the eight months during which irradiations were performed.

RESULTS

The experiments being reported were designed to provide information on the effectiveness of different concentrations of oxygen in altering the induced rate of translocations and recessive lethals; therefore, the choice of the dosage and temperature to be used during irradiation is of importance. An X-ray dosage of 2000 r was chosen for the translocation study because previous work on *D. virilis* (BAKER 1949) had shown that at dosage levels in this region the dosage-frequency curve was still exponential and that complex rearrangements were not too frequent. The choice of irradiating the flies at 5°C is based on the observation that at this temperature the flies are inactive in any concentration of oxygen and thus the oxygen tension within the sperm cells would not be influenced by the activity of the animals.

The data which show that the induction of translocations by X-rays is a function of the oxygen surrounding the flies are summarized in table 1. The

TABLE I

Relationship between oxygen concentration and production of translocations in D. virilis.

(Dosage = 2000 r, temperature = 5–8°C)

Percent O_2 in N_2	Number of individual experiments	Total no. of sperm tested	Total no. of sperm with translocations	Weighted* mean % sperm with translocations
0	3	512	19	3.48
2	3	186	12	6.40
5	5	525	52	9.17
9	3	205	29	12.88
15	5	645	92	14.22
21	5	616	99	15.29
100	3	494	85	17.15

*The results of the individual experiments at a given gas mixture are weighted according to the inverse of the estimated variance of the mean $\left(\frac{100n}{pq}\right)$.

results obtained in the individual experiments at a particular oxygen concentration are not presented in this table since they are too bulky for publication. However, in the last column will be found the weighted mean percentage of sperm which contain translocations for each oxygen concentration. These means are based upon the individual experiments, each of which is weighted according to the inverse of the estimated variance of its mean $(\frac{100n}{pq})$—p and q are estimated from the data. The individual experiments are weighted in this manner to take into account uncontrollable fluctuations, undoubtedly present in different experiments.

It is obvious from the figures in this column that over four times as many of the sperm irradiated in pure oxygen contained translocations as did the sperm irradiated in nitrogen. It is also evident that the number of translocations induced rises quite rapidly with oxygen concentration until the con-

centration reaches about 9 percent, and then any further increase of the concentration has little effect. This relationship can be seen better by an examination of figure 1. The experimental points plotted on this graph are taken from the last column of table 1. The line relating the points at 0, 2, 5, and 9 percent oxygen is the weighted linear regression line (calculated from

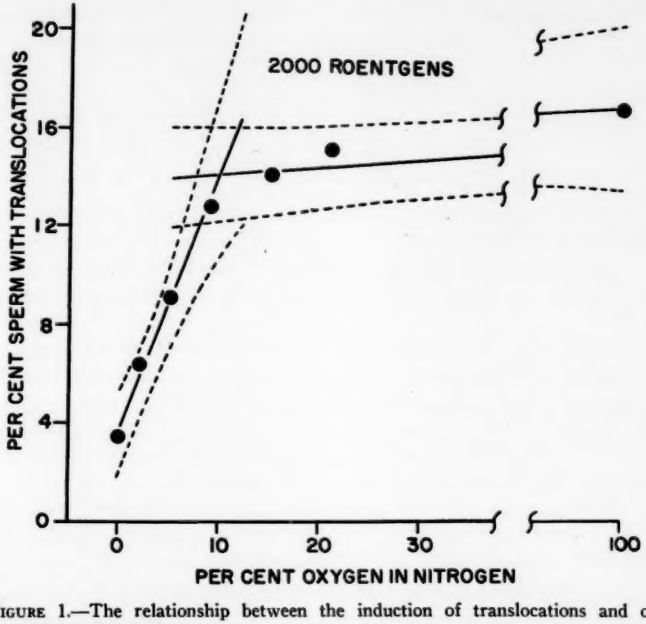

FIGURE 1.—The relationship between the induction of translocations and oxygen concentration in *D. virilis*.

the weighted individual experiments) which best fits these four points according to the method of least squares. Its equation is:

$$\hat{Y}_w = 3.605 + 1.085\,X$$

In a similar manner, the regression line passing through the points at 9, 15, 21, and 100 percent oxygen was calculated to be:

$$\hat{Y}_w = 13.84 + 0.0342\,X$$

From these two equations, it is evident that the change in slope of the oxygen relationship is quite prominent since at lower oxygen tensions the slope is approximately one, while at higher concentrations the slope is almost zero. The dotted lines in this figure enclose plus and minus twice the standard error of the respective weighted regression lines; i.e., the 95 percent confidence limits of the lines. The data are presented in this manner because the exact shape of the empirical curve in the region where the slope changes quite rapidly is not known. It is interesting to note that the two regression lines

TABLE 2

Relation between oxygen concentration and production of recessive lethals in D. melanogaster.

(Dosage = 4000 r, temperature = 5–8° C)

Percent O_2 in N_2	Number of individual experiments	Total no. of X chromosomes tested	Total no. of X chromosomes with lethals	Weighted* mean % X chromosomes with lethals
Control	3	746	1	0.13
0	7	1261	98	7.40
2	6	1228	110	8.62
5	6	951	85	8.38
9	6	581	70	11.43
15	6	908	121	13.06
Air	6	636	82	11.70
100	7	816	125	15.18

*See footnote to table 1.

cross at about 10 percent oxygen; therefore, it seems likely that the induction of translocations is independent of oxygen concentration at 10 percent or above.

In the complementary study on the induction of recessive sex-linked lethals in *D. melanogaster,* the flies were given 4000 r of X-radiation while being maintained at a temperature of approximately 5°C. This dosage was chosen because prior work (BAKER and SGOURAKIS 1950) indicated that one would expect close to a maximum difference between the number of lethals induced in oxygen and nitrogen in the neighborhood of 4000 r.

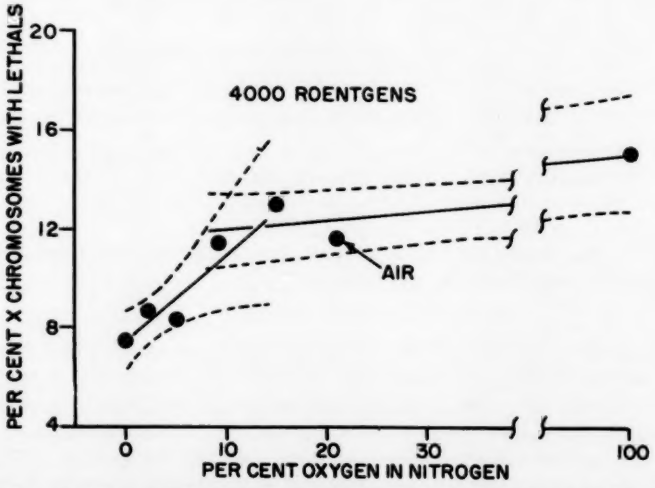

FIGURE 2.—The relationship between the induction of sex-linked recessive lethals and oxygen concentration in *D. melanogaster.*

The results of this study are summarized in table 2. It can be seen from these data that only about twice as many lethals are induced when the flies are treated in pure oxygen as in nitrogen. However, the characteristic increase in the induced recessive lethal rate at the lower oxygen concentrations and its essential independence of oxygen concentration at higher tensions is obvious in this table and in figure 2. The equation of the weighted linear regression of recessive lethal frequency on oxygen concentrations of 0, 2, 5, and 9 percent which is shown in this figure is:

$$\hat{Y}_w = 7.476 + 0.355\,X$$

while the line calculated from the data at 9, 15, air (21), and 100 percent oxygen has the equation:

$$\hat{Y}_w = 11.65 + 0.0352\,X$$

The fact that the two regression lines cross at about 13 percent oxygen indicates that the induced lethal rate is practically independent of oxygen concentration at this point or above.

A comparison of data presented in figures 1 and 2 brings out certain essential facts which should be emphasized. Without further analysis of the data, one might suppose that translocation production in *virilis* is much more sensitive to oxygen concentration than the induction of recessive lethals in *melanogaster*. Also one might conclude that the slope of the translocation curve at low oxygen concentrations is much greater than the slope of the lethal curve at these concentrations. The validity of these conclusions will be discussed later. Finally, it is noteworthy that both of the biological effects become independent of oxygen concentration at tensions higher than about 11 percent oxygen. It has been pointed out previously (HOLLAENDER, BAKER and ANDERSON 1951) that the sharp break between 10 and 15 percent oxygen is probably a distinctive feature of the oxygen effect since it has been reported for chromosome rearrangements in Tradescantia (GILES and RILEY 1950; GILES and BEATTY 1950) and for mitotic inhibition in the neuroblast cells of the grasshopper (GAULDEN and NIX 1950).

Additional data concerning the oxygen effect are obtained from the translocation study by comparing the relative frequency with which different chromosomes are involved in the 388 translocations obtained over the different oxygen concentrations used. In table 3 all the translocations induced in flies irradiated

TABLE 3

Percent of translocations in which the different chromosomes are involved.

Experiments	Chromosomes					Number of translocations
	Y	2	3	4	5	
N_2, 2%, & 5% O_2	27.7	41.0	43.4	41.0	51.8	83
9%, 15%, 21%, & 100% O_2	23.9	53.1	48.8	45.2	43.3	305
All O_2 concentrations	24.7	50.5	47.4	44.3	45.1	388
Baker (1949)—Air	20.7	49.4	48.8	45.5	52.3	808

in nitrogen, 2 and 5 percent oxygen are lumped into one group, while those induced in 9, 15, 21, and 100 percent oxygen are placed in another group. Thus a comparison may be made between the relative frequencies with which the five chromosomes are involved in translocations in the two groups. None of the differences, for a given chromosome, between the percentage involvement in the two groups are clearly significant at the 5 percent level. Thus, there is no evidence that differential breakage of particular chromosomes, e.g., the heterochromatic Y chromosome, is involved in the oxygen effect. These figures also allow a general test of reliability of the data, since they can be compared with the earlier more extensive results of BAKER (1949). As can be seen in table 3, there is no difference, for a given chromosome, between the percentage involvement obtained in this study and the earlier one except for the fifth chromosome. The difference between the frequency of translocations involving chromosome 5 observed in this study and the earlier one is on the border line of significance at the 2 percent level. The reason for this discrepancy, if it is real, is not apparent.

DISCUSSION

One of the primary difficulties involved in studies of the biological action of radiation is to determine exactly what biological process is being affected. In terms of the small production of gross chromosome aberrations when organisms are X-irradiated in low oxygen concentrations, at least three processes could be affected. (1) The breakage process itself might be altered so that, per unit dosage, fewer initial breaks are produced. (2) The process of reunion, *per se*, might be affected so that broken chromosome ends are much more likely to restitute than to join in new arrangements. At higher concentrations the probability of restitution would be less. (3) During irradiation the broken ends could be altered (either when the break was produced or a short period of time later) in such a manner that when reunion took place the ends would be more likely to restitute than to form new arrangements. Interpretations of the oxygen effect in terms of the first process will be called the " breakage " hypothesis, while explanations relating to either of the last two processes will be referred to as the " reunion " hypothesis.

Theoretically, it should be possible to decide which of these processes is being affected if comparable studies were made on the effect of oxygen in influencing the induced rate of point mutations and gross rearrangements in Drosophila. If it is only the process of reunion of broken ends which is affected (whether through changes in the capacity of the ends to reunite or the reunion process itself) and not the number of initial breaks or primary events, then an oxygen effect on induced point mutations would not be expected. The two processes affecting the manner of reunion can possibly be distinguished in Drosophila because they may be separated in time. In sperm, the reunion of the chromosome ends to form new arrangements takes place at the time of fertilization. Presumably, any restitution also occurs at that time, although there is evidence that, under certain conditions, some restitution may take place earlier (KAUFMANN and HOLLAENDER 1946).

From a practical standpoint, it is rather difficult to distinguish in Drosophila between the initial breakage hypothesis and the one attributing the oxygen effect to alteration of the capacity of the broken ends to join in new arrangements, because many of the sex-linked recessive lethals are not true point mutations, but rather are lethals associated with rearrangements. The recovered recessive lethals could have any of the following modes of origin: point mutations not connected with chromosome breaks, mutations which are lethal because of position effects associated with rearrangements (either gross or minute), mutations associated with breaks which act as lethals irrespective of the manner of reunion of the broken ends, and short deletions. The problem as to which process the oxygen is affecting could be resolved if the relative frequency of the various types of recovered lethals were known.

HERSKOWITZ (1951), purposely neglecting lethal position effects caused by minute rearrangements and not differentiating between point mutations associated with breaks and deletions (this class is called "breakage" lethals), has calculated that at 4000 r 20.7 percent of the lethals observed would be caused by point mutations, 6.4 percent by position effects in gross rearrangements, and 72.9 percent by breakage lethals. It can be seen from these figures that, if the oxygen concentration is affecting the reunion process, then only a small portion of the recessive lethals induced in air would be affected by lower oxygen levels—unless small deficiencies make up an appreciable proportion of the breakage lethal class. The question then arises as to how well the data obtained in the studies being reported agree with the expectations based on the breakage or the reunion hypothesis.

The breakage hypothesis

It will be recalled that less than one-fourth as many translocations were induced in nitrogen as in oxygen, while only one-half as many lethals were induced in nitrogen as in oxygen. Now if this difference is to be interpreted on the breakage hypothesis, then it is necessary to take into consideration how translocations and recessive lethals vary with dosage. This is essential because, on the basis of this hypothesis, a reduction in oxygen concentration should produce the same effect as a reduction in dosage. (It should be emphasized that a given reduction in dosage will diminish by a greater extent the number of translocations induced than the same reduction will decrease the frequency of recessive lethals. This is due to the fact that the dosage-frequency relationship for translocations is exponential, while the relationship for recessive lethals is linear.) Such a comparison is possible since the dosage relationship in air for translocations has been determined previously under conditions similar to those in these experiments (BAKER 1949), and the linear relationship between dosage and the production of recessive lethals is a well-established fact. There is good agreement between the percentage of sperm bearing translocations at 2000 r in this study and the earlier one (15.3 and 14.4 percent, respectively). Likewise, the percentage of sex-linked recessive lethals induced in air at 4000 r (11.7 per cent) is in accord with other investigators who find about 2.9 lethals induced per 1000 r (see HERSKOWITZ 1951). From these

dosage curves it is possible to determine an X-ray dosage which produces the same effect as a given concentration of oxygen does at the dosage actually administered to the flies. Figure 3 has been constructed from the dosage curves and this allows for a direct comparison of the two genetic effects on the breakage hypothesis if one assumes that *D. virilis* and *D. melanogaster* sperm are equally sensitive to radiation at similar oxygen levels. The data for recessive lethals, which were obtained at 4000 r, have been translated to 2000 r in order that a comparison of the two curves may be made more easily. This involves the assumption that the magnitude of the oxygen effect is independent of the dose, an assumption which appears to be valid on the basis of previous work (BAKER and SGOURAKIS 1950).

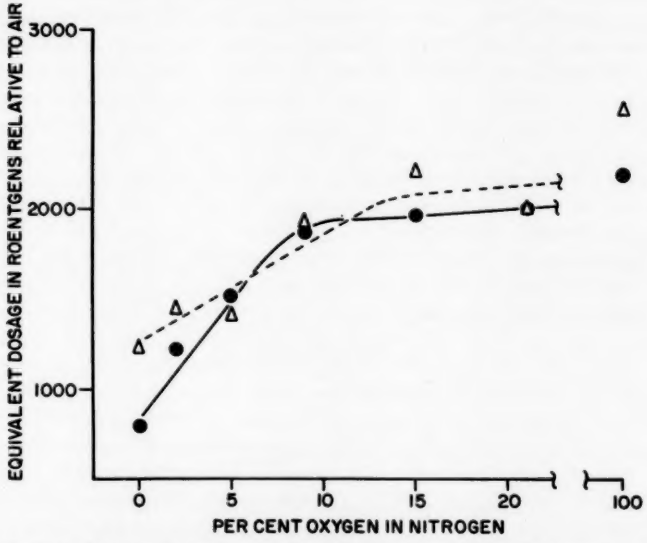

FIGURE 3.—The oxygen effect on sex-linked recessive lethals and translocations in terms of the dosages relative to air which are equivalent to the given oxygen concentrations. See text for full description. △ = Recessive lethals; ● = Translocations.

There are certain features to be noted about these curves. If the breakage hypothesis were true, the two curves should coincide; i.e., the magnitude of the oxygen effect would be the same for lethals as for translocations. It would appear from this figure that a lowering of the oxygen concentration produces a greater reduction in effective dosage in the case of translocations. For example, a decrease of the tension from 21 percent to zero reduces, in effect, the dosage by only 760 r in the case of lethals, but by 1200 r with translocations. However, it should be noted that each of these curves is subject to sampling errors in both their concentration-frequency and their dosage-frequency components; thus, the apparent difference between them may be spurious. Al-

though these data are not sufficient to establish beyond doubt that the breakage hypothesis is not valid, they do raise the interesting possibility that other processes may be involved.

Also in Tradescantia it has not been possible to resolve the problem at the present time. GILES and RILEY (1950) have shown that, by quickly changing the oxygen concentration immediately after irradiation when some breaks would presumably still remain free, the reunion process itself was not affected. Although they appear to favor the initial breakage hypothesis, they point out that lowered oxygen tensions could affect the chromosomes at the time of breakage and that, when rejoining takes place, the ends may be more likely to restitute.

The reunion hypothesis

The possible difference, noted previously, between the effectiveness of oxygen in changing the induced mutation rate as compared with the chromosome aberration rate is not unique to Drosophila. HAYDEN and SMITH (1949) report that over four times as many chromosome aberrations (chromatinic bridges) are observed in root tips formed from barley seeds irradiated (1200 r of X-rays) in air as compared to vacuum, while fewer than twice as many mutations were observed at the higher oxygen concentration. The magnitude of this difference in barley seems to be in remarkable agreement with the Drosophila data being reported. Since there is the possibility that this difference cannot be explained on the breakage hypothesis, can it be interpreted on the basis of differential reunion? In an attempt to answer this question, let us return to the data presented in figures 1 and 2. On the reunion hypothesis, the difference in oxygen effect between the translocation and lethal data would be a real difference, since the dosage relationships of the two genetic effects would not be involved. A fruitful approach for the understanding of this difference is based on the observation that, the effect in both cases is limited by a particular external oxygen concentration of about 11 percent (undoubtedly corresponding to a definite oxygen tension within the sperm). Therefore, the magnitude of the effect is determined by the slope of the oxygen relationship up to this limiting point. The slope of the translocation linear regression line was found to be 1.1 while the line for lethals has a slope of 0.36. The lower slope, in the case of lethals, could be accounted for on the reunion hypothesis of the oxygen effect because only a portion of the lethals are associated with the manner in which broken chromosome ends rejoin; while in the case of translocations, the induction of any one is dependent upon the reunion process. If one accepts the reunion hypothesis and if it is assumed that the slope of 1.1 indicates the manner in which the frequency of gross rearrangements increases with oxygen concentration, then it can be calculated that, in order to produce a slope of 0.36, 32.7 percent of the recovered recessive lethals would have to be lethal because of their association (position effects or deletions) with gross arrangements. This figure seems too high to be in accord with the proportion, cited above, which is postulated by HERSKOWITZ. However, as has been

pointed out by MULLER (1950), too much reliance should not be placed on the exactness of calculations similar to those of HERSKOWITZ since they are based on certain assumptions, the validity of which has not been tested, concerning the manner of reunion of broken ends. Practically nothing is known about this process in Drosophila.

Rather strong support for the reunion hypothesis comes from studies of SCHWARTZ (1952) in maize. Pollen was X-irradiated in air and in nitrogen and, by use of suitable markers, it was possible to determine in the endosperm the relative proportion of loss of the markers and mosaics. The mosaics arise when the chromosome in the pollen is broken distal to the marked loci and no restitution follows, while the losses are formed from internal deletion followed by rejoining, or from terminal deficiencies. He finds a high proportion of mosaics among the aberrant kernels coming from pollen treated in air but only a very small proportion in the kernels from the nitrogen-treated pollen. Therefore, it appears that in maize pollen rejoining of the broken ends is favored if the oxygen concentration is kept low during irradiation. It is difficult to see how a decreased number of breaks formed in nitrogen could cause a shift in the ratio of mosaics to losses in the direction found.

CONCLUSIONS

The situation may be summarized by stating that the data presented are not in disagreement with expectations based on the breakage hypothesis of oxygen action but there remains the possibility that the apparent difference in magnitude of the oxygen effect between translocations and lethals is real. Neither is the agreement completely satisfactory for the reunion hypothesis, since a larger proportion of the recovered recessive lethals, than we are aware of at present, would have to be caused by chromosome breaks and rearrangements which were lethal because of the way the ends rejoined. It should also be noted that the wide variety of radiobiological effects which are altered by low oxygen tensions also militates against this hypothesis. Perhaps the active substances formed by the interaction of oxygen and X-rays in protoplasm alter both the initial number of breaks and the manner of reunion.

It is now apparent that in Drosophila a solution to the problem under discussion will be difficult to obtain until at least two questions have been satisfactorily answered. In the first place, information as to the qualitative constitution of the recessive lethals recovered at various dosages is necessary. Secondly, the relationship between dosage and the amount of the substance, which is formed by the interaction of oxygen and X-rays and which either breaks the chromosome or affects the reunion of the broken ends, would be helpful.

SUMMARY

Translocations were induced in *Drosophila virilis* sperm by exposing to 2000 r of X-rays adult males maintained in various concentrations of oxygen during treatment. Less than one-fourth as many translocations are induced in the absence of oxygen (in nitrogen) as is the case when the males are irradi-

ated in 100 percent oxygen. Studies on the production of sex-linked recessive lethals in *D. melanogaster* indicate that about one-half as many lethals are induced by 4000 r of X-rays when the males are exposed in a nitrogen environment as compared with pure oxygen.

For the induction of both translocations and recessive lethals, the shape of the curves relating the genetic effect to oxygen concentration is strikingly similar. At low oxygen concentrations, small changes in the oxygen tension cause a rapid rise in the amount of genetic effect produced until a concentration of about 11 percent is reached. At this point the curves break sharply and further increase in the amount of oxygen has little or no effect on the frequency with which translocations or recessive lethals are induced by a given dosage of X-radiation.

On the basis of these data, it is still an open question in Drosophila as to whether a low oxygen tension is reducing the initial number of primary events produced by the radiation or whether it is influencing the reunion of broken chromosome ends such that restitution is favored over recombination.

ACKNOWLEDGMENTS

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VIABLE DEFICIENCY-DUPLICATIONS FROM A TRANSLOCATION IN GOSSYPIUM HIRSUTUM [1]

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GOSSYPIUM hirsutum L., which includes the Upland varieties of cultivated cotton, is known to be an allotetraploid species with 26 pairs of chromosomes. Thirteen relatively large pairs (A genome) are closely homologous with those of the diploid Asiatic cultivated cottons, and thirteen relatively small pairs (D genome) with those of wild New World diploid species of Gossypium (for recent review see BROWN 1951). Several pairs of genes are known to be linked in *G. hirsutum*, but these have not been assigned to specific chromosomes. Beyond this, little is known of the cytogenetic anatomy of *G. hirsutum*. At present, an attack on this problem is being made by various methods, including cytological and genetic studies of trisomic, tetrasomic, monosomic and translocation lines.

The present paper deals with a translocation from which not only normals and heterozygous translocations are recovered on backcrossing to normal lines, but also an array of duplication and deficiency types. This translocation is of interest not only because of its cytological behavior, but also because some of its derivatives should prove useful in further linkage studies.

The original translocation appeared in a plant (designated 2B-1) grown from seeds of a commercial variety of cotton, Acala 911, which were exposed to gamma radiation of unknown intensity and duration at the time of the explosion of the first atomic bomb at Bikini (Operation Crossroads). This material was obtained through the courtesy of the U. S. Department of Agriculture in cooperation with the Naval Medical Research Section, Joint Task Force One.

Cytological analyses were carried out for the most part on field-grown plants, and selected plants were transplanted to the greenhouse for crossing and selfing. Buds were fixed in a mixture of 3 parts glacial acetic acid: 7 parts 95 percent alcohol, and pollen mother cells were smeared in iron-acetocarmine for the study of meiosis. Figures for frequencies of different meiotic configurations are taken from 1951 analyses only.

THE HETEROZYGOUS TRANSLOCATION

A description of chromosome configurations in 2B-1 is given by BROWN (1950). Configurations in recovered heterozygous translocations agree with

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this description. Figure 1 shows the frequency of the various configurations and diagrams of their presumed structure. So far as can be seen at metaphase I in the pollen mother cells (the earliest stage of meiosis which can be studied readily in cotton), the translocation behaves as if it were nonreciprocal. Most of the long arm of a large chromosome with subterminal centromere (chromosome 2) appears to have been translocated terminally to one arm of a second large chromosome with approximately median centromere (chromosome 1). The characteristic configuration is a bivalent with an attached chain of two. A chiasma is usually formed proximal to the point of attachment of the long fragment (designated "A" for "attached fragment"), and the broken arm of the centric fragment ("C") is always free. Since there is no evidence that broken ends of chromosomes ever unite with unbroken ends, it must be assumed that a minute terminal segment of chromosome 1 (designated "x") was broken off, but we have at present no cytological evidence as to whether x was translocated to the broken end of C, or lost, which would require healing of the broken end of C (see McCLINTOCK 1941).

For descriptive purposes, therefore, it will be convenient to treat the translocation as if it were nonreciprocal, and to ignore the questionable status of the x segment. Some indirect evidence suggests, however, that the x segment contains functional chromatin, and that the translocation is actually reciprocal but very unequal.

Plants heterozygous for the translocation are normal in appearance, so that cytological analysis is necessary for their identification. No controlled comparison of their fertility with that of normal plants has been undertaken, but well-filled bolls are set following both self and cross pollination, indicating that at least ovule fertility is approximately normal. The studies reported here were carried out in crosses using the translocation heterozygote as the pistillate parent; hence pollen fertility has not been tested.

Recovery of deficiency and duplication types

The original translocation (2B-1) was brought into the greenhouse in 1947 and allowed to set seed. In 1949 a progeny of 13 plants was grown from the seed, of which 11 were examined cytologically. In addition to normals and translocation heterozygotes, several plants appeared which could not be assigned with confidence to either of these classes. However, it was thought possible, though unlikely, that 2B-1 was a chimera of several different cytological types produced by the radiation (BROWN 1950). Two plants with heterozygous translocation configurations of the 2B-1 type, and a third unanalyzed plant (subsequently shown to have been of the same type) were crossed as pistillate parents with a cytologically normal genetic marker stock (SL7-9) carrying five independent dominant characters.

Small progenies were grown from these crosses the following summer, and meiosis was studied in fifteen plants. It was again apparent that in addition to normals and translocation heterozygotes, other cytological types were being recovered. The extreme inequality of the interchanged arms fortunately made

it possible to distinguish each of these types with ease at metaphase I. The meiotic configurations of 14 of the plants agreed with those expected if maternal gametes were being recovered from both disjunction and nondisjunction of the translocation IV. The fifteenth plant was one of the types expected if a maternal gamete resulting from numerical (3 : 1) nondisjunction were recovered. These results, the gametes involved, the identifying meiotic configuration, and the designation adopted for the resulting genotype are shown diagrammatically in figure 2.

One plant was analyzed from a selfed progeny of a fourth plant of the 1949 progeny heterozygous for the same translocation. This plant had 26 II plus a minute I. Since this type is also expected from numerical nondisjunction of the IV, the plant was considered to be of the constitution $2n + C$.

Two of the heterozygous translocations recovered in 1950 were outcrossed to recessive normal lines, and 68 plants were grown from these crosses in 1951. Meiosis was studied in 58 of these. The types found agreed with those of 1950, with two exceptions: (1) The $2n - C$ and $2n + C$ types did not reappear. (2) One plant of the $2n + A$ type was monosomic for one chromosome not involved in the translocation. This plant will be ignored in the present discussion. The distribution of the remaining 57 plants among the four expected types is shown in figure 2. The frequency of the four types (26 II, IV, $2n - A$, $2n + A$) did not differ significantly from equality ($\chi^2 = 1.88$, $P = 0.50 - 0.70$, 3 d.f.).

Cytological considerations

The majority of heterozygous translocations studied in plants have been characterized by partial sterility, in most cases approximating 50 percent (cf. RILEY 1948, pp. 402–411). This result is generally attributed to nonfunctioning of the gametes (duplications, deficiencies, or both) resulting from nondisjunctional separation of the chromosomes at meiosis. Deviations above or below the 50 percent level of fertility have generally proved to be due to a preponderance of metaphase I orientations on the spindle resulting in an excess of disjunctional or nondisjunctional separations, respectively.

The present translocation differs markedly from the usual case, therefore, since at least the female gametes resulting from disjunction and from nondisjunction are recovered with approximately equal frequency. This implies either that (1) there is a preponderance of nondisjunctional separations but selection favors the disjunctional gametes to just such an extent that they are recovered as frequently as the $n - A$ and $n + A$ gametes, or (2) that there is no selection against $n - A$ and $n + A$ gametes and the meiotic orientation assures random assortment of the chromosome segments involved.

A study of pairing and orientation at metaphase I in 139 pollen mother cells (combined data from 11 heterozygous translocation plants) indicates that the latter alternative is the correct explanation. The data are shown in figure 1. It is deduced from these data that the equality of disjunctional and nondisjunctional gametes ensues from the following considerations. The break in the

arm of chromosome 2 which led to the C fragment was close to the centromere, proximal to the segment in which chiasmata are formed. In 566 cells analyzed from 59 $2n-A$ plants, as well as in the 139 from the heterozygous translocation, the broken arm of C was always free. But the unbroken arm of C was paired with the short arm of chromosome 2 about 79 percent of the time (fig. 1 a, b, c, d, f). Hence if C and chromosome 2 disjoin at all, the first division must be reductional for the broken arm as well as for the centromeres. On the other hand, the point of attachment of A on chromosome 1 appears to be distal to the chiasma-forming region. In 98.65 percent of the cells (all but two, fig. 1 c, d) an interstitial chiasma was present in this arm. As pointed out by SANSOME (1933) and SUTTON (1935), this assures that the first division is almost always equational for the A segment, and the second division

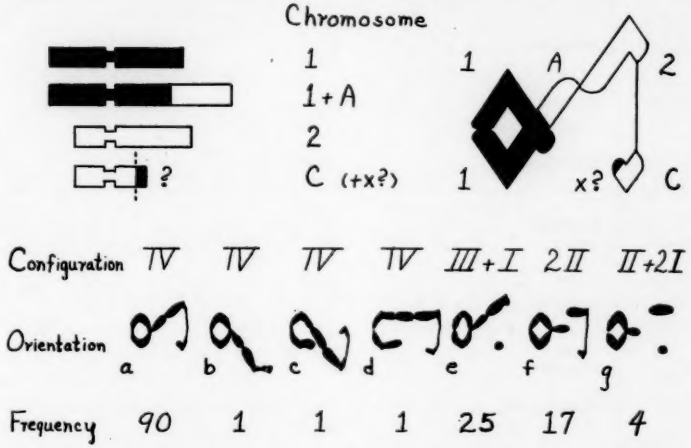

FIGURE 1.—Structure of the heterozygous translocation and frequencies of the various orientations observed at metaphase I in 139 pollen mother cells (1951).

reductional (assuming that the single visible chiasma represents one and only one crossover).

All individual meioses in which the first division is reductional for C and equational for A would result in two "disjunctional" and two "nondisjunctional" gametes, regardless of the orientation of the centromeres at metaphase I. In the sample studied, 107 cells (76.3 percent) fulfilled these requirements (fig. 1 a, f).

If C and chromosome 2 pair but no chiasma is formed between A and the centromere of chromosome 1, the first division is reductional for both A and C, and disjunction depends upon the metaphase I orientation of the centromeres of chromosome 2 with respect to those of chromosome 1. Two cases of this sort were seen in the heterozygous translocations. In one (fig. 1 c), separation was proceeding disjunctionally, and in the other (fig. 1 d), nondisjunc-

tionally. Hence, a total of 109 cells (78.4 percent) recorded should give a 1:1 ratio of disjunctional and equal nondisjunctional gametes.

The remaining 30 configurations (21.6 percent) assure ultimate disjunction of chromosomes 1 and 1 + A, but allow chromosomes 2 and C to pass to either the same or opposite poles (fig. 1 b, e, g). If this occurs at random, half should give disjunctional and nondisjunctional gametes in a ratio of 1:1. The other half (excluding loss of the univalent chromosomes) should give only gametes of the numerical nondisjunctional classes ($n-C$, $n+C$, $n+C+A$, $n-1$). These four types of gametes together should therefore constitute about 10.8 percent of the total, while the remaining types should make up about 89.2 per-

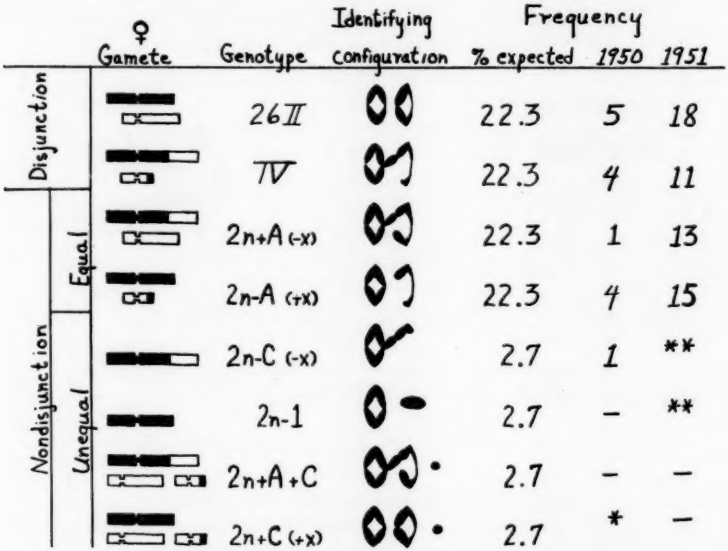

FIGURE 2.—Results of the cross, translocation heterozygote female X normal male. *One recovered from IV selfed. **Recovered from $2n - C$ selfed.

cent of gametes, equally distributed among two disjunctional and two nondisjunctional classes. These expectations are shown in figure 2. It must be pointed out that these studies of meiosis were carried out on pollen mother cells, while the gametes actually tested resulted from meiosis in the embryo sac mother cells. It will be noted, however, that at least the disjunctional and equal nondisjunctional classes expected and recovered are in close agreement. The numerical nondisjunctional gametes have not been recovered quite so frequently as expected from the cytological estimate. Actually only one has been found in backcrosses of the heterozygous translocation to normal lines, among a total of 72 plants, whereas about seven or eight might have been expected. This discrepancy might be due to sampling error, to loss of univalents at anaphase I, to a slightly different pattern of frequencies of the various configura-

tions in the embryo-sac mother cells, or to a combination of these and other factors. It is possible that the numerical nondisjunctional gametes suffer from some selective disadvantage which reduces their rate of recovery. That the zygotes they enter are viable, however, is shown by the fact that three of the four genotypes have been recovered either from the heterozygous translocation itself or from its $2n-C$ derivative (fig. 2).[2] These three types are vigorous and fertile, not phenotypically different from the more common genotypes.

TRANSMISSION OF THE UNBALANCED COMPLEXES

It will be seen from figure 2 that two of the nondisjunctional complexes recovered from the heterozygous translocation are deficiencies for complementary portions of chromosome 2; $2n-A$ is monosomic for most of the long arm, while $2n-C$ is monosomic for the centromere region and the short arm. If the monosomic condition were transmitted, these types could be used in testing for the location of marker genes not only upon chromosome 2, but upon specific portions of it. Cytological classification of the offspring of these and other unbalanced types would also serve as an independent test of our

TABLE 1

Transmission of the $2n-A$ condition.

Types expected	Number of plants analyzed from		
	$2n-A$ selfed	$2n-A♀ \times$ normal	normal $\times$ $2n-A♂$
26 II	51	28	32
$2n-A$	38	7	0
$2n-2A$	0	0 (not expected)	0 (not expected)
Total	89	35	32

assumptions regarding the constitution of the parent plants, since the cytological classes expected from each are different.

Accordingly, progenies from $2n-A$, $2n-C$ and $2n+C$ plants recovered in 1950 were grown in 1951. Rather extensive analyses of meiosis were made, since information on pairing and metaphase I orientation of the various chromosome segments has important implications for the further use of these types in cytogenetic studies.

Transmission of the $2n-A$ condition

Three of the $2n-A$ plants were selfed, and outcrossed as both seed and pollen parent. As can be seen from table 1, plants deficient for A were readily recovered through the ovule, and no evidence was obtained that $n-A$ pollen functioned. A discrepancy appears when the frequencies of $2n-A$ plants in selfed and outcrossed progenies are compared, however: recovery was appreciably higher in the selfed progeny. No explanation is offered for this fact at present.

[2] In the summer of 1952, the fourth type, $2n+A+C$, was recovered from the heterozygous translocation selfed.

Among 566 pollen mother cells from a total of 59 $2n-A$ plants, the C fragment and chromosome 2 failed to pair 21 percent of the time. Provided the univalents are not lost but separate at random, about 5 percent each of $n+C$ and $n-1$ gametes should be produced by $2n-A$ plants. These types have not been recovered from $2n-A$. It is probable, however, that occasional $2n+C$ and $2n-1$ plants can occur, since both $n+C$ and $n-1$ eggs are known to have functioned in other progenies.

Transmission of the $2n+C$ condition

In 1951 a small progeny was grown from selfed seeds of the $2n+C$ plant. Only ten plants were analyzed, of which six had 26 II and four were $2n+C$. Plants with two C fragments were not found. It is likely that the $n+C$ gametes were recovered from the ovules and that only n pollen functioned, although data from trisomic studies in which tetrasomics were recovered (BEASLEY and BROWN 1943; BROWN 1949, and unpublished) make it probable that $2n+2C$ plants could be obtained occasionally.

TABLE 2

Transmission of unbalanced gametes from $2n-C$ selfed.

	Constitution of ♀ gamete	Number recovered
Types expected if only n pollen functioned		
26 II	n	15
$2n-C$	$n-C$	9
$2n+A$	$n+A$	7
$2n-1$	$n-1$	7
Other types found (expected if $n+A$ pollen also functioned)*		
$2n+2A$	$n+A$	2 (dwarf)
$2n+A-C$	$n-C$	2 (dwarf)

*Two additional dwarf plants were not analyzed.

In a total of 20 $2n+C$ pollen mother cells, the C fragment was paired in a III 50 percent of the time, in comparison with IV and $2n-A$ plants, in which C was paired 77–79 percent of the time. Although C can pair half the time even when two normal chromosomes 2 are present, no case was seen in which C was paired with one normal chromosome and the other normal chromosome was unpaired. Probably only n and $n+C$ gametes are to be expected from $2n+C$.

Transmission of unbalanced types from $2n-C$

The $2n-C$ plant was selfed and a progeny of 49 plants was grown. Meiosis was studied in 42 of these. Of the analyzed plants, 38 conformed cytologically with the types expected if all four kinds of female gametes (n, $n-C$, $n+A$, $n-1$) functioned and were fertilized with normal n pollen (table 2). Although there was a preponderance of normal (26 II) types, it will be seen that the

other three classes were recovered with appreciable frequency. As in progenies from the heterozygous translocation and from $2n - A$ plants, the cytologically unbalanced plants were phenotypically normal and fertile.

Six plants in the progeny of 49 plants did differ morphologically from the rest, however. These were very small and slow growing, with slender stems reaching a maximum height of less than a foot during the growing season. One succumbed after setting a small boll. The other five were transplanted to

FIGURE 3.—Metaphase I in a dwarf, $2n + A - C - 2x$ plant, showing III composed of two $1 + A - x$ chromosomes and one chromosome 2.

FIGURE 4.—Metaphase I in $2n + A - C - 2x$ plant, showing a II of two $1 + A - x$ chromosomes, and an unpaired chromosome 2. Note failure of the chiasma to terminalize in the $1 + A$ chromosomes, even when these are paired as a II.

FIGURE 5.—Metaphase I in a dwarf, $2n + 2A - 2x$ plant, showing IV of two $1 + A - x$ chromosomes and two chromosomes 2.

the greenhouse in October, where they produced new growth but remained slender and unthrifty. All nevertheless proved to be fertile. Meiotic configurations were analyzed in four of these plants. Two had a pair of $1 + A$ chromosomes plus a normal chromosome 2 pair (fig. 5) and hence were tetrasomic for the A segment ($2n + 2A$). The other two also had a pair of $1 + A$ chromosomes, but only a single (normal) chromosome 2 (fig. 3, 4). They are therefore trisomic for the A segment, but monosomic for the centromere region of chromosome 2 ($2n + A - C$).

These plants provide the first instance so far obtained of transmission

through pollen of unbalanced gametes from the 2B-1 translocation. The $2n+2A$ plants can have been produced only by the functioning of $n+A$ pollen. It is most likely that the $2n+A-C$ plants were produced by $n+A$ sperm fertilizing $n-C$ eggs, since trisomics are often transmitted through pollen in cotton, while only one case of male transmission of a monosomic has been found (unpublished data).

The dwarf plants are the only phenotypically abnormal types so far recovered from 2B-1. Neither $2n-C$ nor $2n+A$ plants are dwarf. Tetrasomy sometimes produces abnormal phenotypes in cotton, but only the $2n+2A$ plants are tetrasomic (for the A segment). But both dwarf types are homozygous deficiencies for the hitherto neglected x segment of chromosome 1. It seems probable that the dwarf phenotype is due to the homozygous deficiency.

If this interpretation is correct it may enable us to determine whether the x segment was translocated to the broken end of C, or deleted. If the former, the homozygous translocation should be normal; if the latter, it should also be dwarf. Selfed progenies of the heterozygous translocation have not been studied since the realization that all of the cytological classes could be distinguished. It is probable, however, that at least one homozygous translocation was recovered in the 1949 progeny from 2B-1. This plant was characterized by a large bivalent with an unterminalized chiasma in the longer arm, like the $1+A$ II shown in figure 4, and a second very small, attenuated bivalent, probably the C II. The plant in question was phenotypically normal, which makes it seem probable that the translocation was truly reciprocal. Further study of this point is not yet completed.[3]

TERMINALIZATION OF CHIASMATA

It has long been questioned whether the single terminal or nearly terminal chiasma per arm characteristic of metaphase bivalents in Gossypium represents a low original chiasma frequency, or well-advanced terminalization. Prophase stages are unfavorable for direct investigation of the question, and so far no three-point linkage groups have been available for an estimate of double crossover frequencies by genetic means.

Observations incidental to the present study suggest that the single interstitial chiasma seen proximal to A at metaphase represents the maximum number formed earlier.

At metaphase I in plants of the classes IV, $2n+A$ and $2n-C$, which are heterozygous for chromosome $1+A$, the interstitial chiasma proximal to A

[3] Since the manuscript was submitted for publication, four plants homozygous for the translocation have been identified cytologically. These four plants are phenotypically normal. In addition, from selfing the heterozygous translocation a plant has been recovered which shows chromosome configurations identical with those found in the $2n+2A-2x$ dwarfs (a pair of $1+A$ chromosomes and a pair of normal chromosomes 2), except that it has in addition a C chromosome ($2n+2A+C-x$). This plant is also phenotypically normal. These findings support the conclusion that the translocation was reciprocal, and that the dwarf habit of $2n+2A-2x$ and $2n+A-C-2x$ plants is due to the homozygous deficiency for the x segment.

clearly fails to terminalize past the point of translocation. Even when the A arm is not paired with chromosome 2, a heteromorphic bivalent with one chiasma in each arm can often be identified by the long, single A arm projecting beyond the chiasma. In cells in which no chiasma is present proximal to A, the point of attachment is usually marked by a slight constriction or faintly stained region.

Failure of the chiasma to terminalize in heterozygotes for A is probably not due to mechanical difficulties inherent in the unequal length of the paired arms, or to change of homology (cf. DARLINGTON 1937, p. 510). Terminalization also fails in $2n + 2A$ and $2n + A - C$ plants, where both chromosomes 1 have A segments and are therefore of equal length and presumably homologous throughout. In a total of 90 pollen mother cells, the two A arms could be distinguished with certainty in 77 cells. The majority showed the unterminalized chiasma proximal to A. Those which lacked it showed the constriction at the point of attachment.

It appears, then, that the region of breakage and attachment of A in chromosome 1 serves to prevent pre-metaphase terminalization of chiasmata proximal

TABLE 3

Segregation of five characters in a selfed progeny of $2n - A$ plants from the cross recessive heterozygous translocation X dominant normal SL7-9.

Cytological type	Plant color		Leaf shape		Petals		Lint		Seed	
	Red	Green	Okra	Broad	Spot	No spot	Brown	White	Naked	Fuzzy
26 II	41	10	42	9	35	16	39	12	33	18
$2n - A$	27	11	25	13	31	7	28	10	22	16
Total	68	21	67	22	66	23	67	22	55	34

Expected 3:1 ratio = 66.75:22.25.

to it. (No evidence was seen that anaphase separation is obstructed, however). If this is true, it follows that not more than one chiasma is characteristic of the arm at prophase, since no more than one metaphase chiasma was ever seen proximal to A (in a total of about 575 cells from IV, $2n + A$, $2n - C$, $2n + 2A$ and $2n + A - C$ plants combined).

TESTS WITH GENETIC MARKERS

The transmission of an appreciable number of deficiency types $2n - A$ and $2n - C$, together with the $2n - 1$ plants recovered from $2n - C$, makes it possible to detect the presence or absence of marker genes on chromosome 2 by a modification of the monosomic method (CLAUSEN 1941; CLAUSEN and CAMERON 1944).

The three $2n - A$ plants from which the 1951 selfed progeny was grown were heterozygous for five dominant genes which entered the cross from the cytologically normal parent. If any of these five genes were located on the A segment, the selfed progeny should fail to segregate the recessive phenotype. The genetic data are shown in table 3. The progeny was segregating for all

five characters, with both 26 II and $2n - A$ classes including both dominant and recessive phenotypes for all five characters. We may therefore conclude that none of the loci marked in SL7-9 is located on the A segment.

The $2n - C$ plant from which the 1951 selfed progeny was grown was similarly heterozygous for SL7-9. Reference to figure 2 and table 2 will show that if one of the five genes were located on C, none of the F_2 plants should show the recessive phenotype, since the only segment homologous with C present in the parent was in the unbroken chromosome 2 from SL7-9.

Despite the rather small population, recessives were recovered in every cytological class for each character, except no petal spot in the $2n - C$ class, and all pairs of characters showed a good fit for a 3 : 1 ratio for the population as a whole (table 4). Therefore none of the five loci marked in SL7-9 is on the C segment of chromosome 2. Since C and A together should equal a whole chromosome 2, it follows that none of the markers is on any part of chromosome 2, a conclusion borne out by recovery of recessives for each gene pair in the $2n - 1$ class.

TABLE 4

Segregation of five characters in a selfed progeny of a $2n - C$ plant from the cross recessive heterozygous translocation X dominant normal SL7-9.

Cytological type	Plant color		Leaf shape		Petals		Lint*		Seed	
	Red	Green	Okra	Broad	Spot	No spot	Brown	White	Naked	Fuzzy
$2n - C$	6	3	7	2	9	0	7	2	5	4
$2n - 1$	6	1	4	3	5	2	5	2	5	2
$2n + A$	6	1	6	1	4	3	5	2	5	2
26 II	11	4	13	2	12	3	11	3	11	3
Total	29	9	30	8	30	8	28	9	26	11

Expected 3 : 1 ratio = 28.5 : 9.5.
*One 26 II plant not recorded.

It is now desirable to test whether any of the markers of SL7-9 is located on chromosome 1. It may be said at once that none is on the terminal (x) segment of chromosome 1, since one of the $2n + 2A$ plants, a homozygous deficiency for the minute distal segment, nevertheless had all five dominant characters. No types deficient for other parts of chromosome 1 have been recovered from the translocation, and pairing relations make it unlikely that they can be recovered with ease. The possibility remains of determining whether any of the five genes in question is located on chromosome 1 close enough to the point of breakage to show linkage with the attached A segment from chromosome 2.

The heterozygous translocations recovered in 1950 were heterozygous also for the dominant markers carried by SL7-9 (red body, okra leaf, petal spot, brown lint, naked seed). These were backcrossed to a cytologically normal recessive line to produce the 1951 progeny. Ignoring the single plant which was monosomic for a third chromosome not involved in the translocation, 57 analyzed plants remain, distributed equally in four cytological classes (fig. 2). Of these four classes, two (IV and $2n + A$) are heterozygous for altered chro-

mosome 1 bearing the A segment, and two (26 II and $2n-A$) are homozygous for normal chromosome 1. These two combination cytological classes do not deviate significantly from a 1 : 1 ratio (table 5). As before, the dominant markers entered the cross with the normal chromosomes. A segregation of 1 : 1 is expected for each pair of markers in the backcross progeny, and none of the ratios differed significantly from the expectation.

Therefore, if none of the genes in question shows linkage with the point of translocation, the parental versus recombination ratio should also be 1 : 1. All of the linkage χ^2's are nonsignificant except that for the segregation of red and green (table 5). For the red-green pair, the χ^2 for parental versus recombination types is 5.07, P = .02–.05, which would seem to indicate that red and the point of translocation are not independent. But, as shown in table 5, the deviation is in the opposite direction from that expected if red-green were linked with the point of translocation; there is an excess of recombination rather than of parental types (20 parental : 37 recombination plants).

TABLE 5

Segregation of characters in a backcross progeny of a heterozygous translocation heterozygous for SL7-9.

Character pair	Parental classes			Recombination classes		
	IV & $2n+A$ recessive	26 II & $2n-A$ dominant	Total	IV & $2n+A$ dominant	26 II & $2n-A$ recessive	Total
Red vs. green*	9	11	20	15	22	37
Okra vs. broad	12	16	28	12	17	29
Petal spot vs. no petal spot	13	17	30	11	16	27
Brown vs. white	9	18	27	15	15	30
Naked vs. fuzzy	15	18	33	12	12	24

Expected parental vs. recombination: 28.5 : 28.5

	χ^2	P
*IV & $2n+A$ vs. 26 II & $2n-A$	1.421	.20–.30
Red vs. green	0.438	.50–.70
Parental vs. recombination	5.070	.02–.05
Total	6.969	.05–.10

If the significant χ^2 for linkage is assumed to indicate the location of the red locus on chromosome 1, the crossover percentage between red and the point of translocation is estimated to be 64.9 ± 6.3. Even with a sample of only 57 plants, it may be concluded that crossing over exceeded 50 percent, unless a 1 in 100 mischance in sampling occurred. It is planned to repeat the experiment with larger numbers to see if these puzzling results can be repeated, and if so, to obtain a more reliable estimate of the crossover frequency. While it has been shown that crossing over in excess of 50 percent can occur (FISHER and MATHER 1936; FISHER 1948), such cases appear to be rare. The cytological observations discussed above fail to suggest a meiotic basis for it in the present case, since they seem to indicate that only one chiasma is formed between A and the centromere of chromosome 1, and that the chiasma represents the usual single crossover between two of the four chromatids.

It may be mentioned in passing that the location of red on chromosome 1 is

not in accord with the evidence that the only anthocyanin locus (R_1) known to give red plant body in *G. hirsutum* is in the D genome (SILOW 1940, 1946; HARLAND and ATTECK 1941). The chromosome 1 pair forms one of the larger bivalents at metaphase I, the cytological inference being that it is a bivalent of the A genome.

DISCUSSION

In summary, a total of seven nondisjunctional complexes has been recovered from the 2B-1 translocation and its derivatives. One of these is a simple monosomic ($2n-1$). Three are in reality deficiency-duplication types, if the minute x segment is taken into account: $2n+A$ $(-x)$, $2n+2A$ $(-2x)$, and $2n+A-C$ $(-2x)$. If the translocation is indeed reciprocal, as seems likely, the $2n-A$ type must also actually be a deficiency-duplication ($2n-A+x$), while $2n+C$ is actually a double duplication, $2n+C+x$, and $2n-C$ is a double deficiency, $2n-C-x$. Both duplications and deficiencies for C, A and x are functional in the ovules, while duplications of A, and probably also of C and x, are pollen-viable. In addition, deficiencies of x are to some extent transmissible through the pollen. Since all seven genotypes are viable and fertile, it seems highly probable that several additional genotypes are recoverable by proper manipulation, if the need arises. In addition, there is a high degree of likelihood that the homozygous translocation may be established for use in further studies.

The recovery of $2n+2A-2x$ and $2n+A-C-2x$ plants demonstrates for the first time in cotton, the pollen transmission of a small deficiency and its recovery in the homozygous condition. STEPHENS (1950), in a discussion of the possible role of cryptic structural hybridity in the differentiation of closely related species of Gossypium, points out that two assumptions are necessary to the theory: "(a) that small deficiencies would not always behave as pollen lethals and (b) that crossing-over would occur frequently between only partially homologous chromatids." The pollen transmission of deficiencies for x demonstrates that the first condition can exist in cotton. Moreover, this deficiency is of a size to render plausible its classification as a cryptic structural change, since its presence or absence cannot be detected except by inference at the meiotic stages which can be studied critically. In a formal sense, the pairing relations in the translocation and its derivatives, in particular the regular formation of the interstitial chiasma proximal to A, also satisfy the second condition, since a normal-appearing, two-chiasma bivalent can be formed even though the ends of the paired arms are not homologous. While estimation of the importance of cryptic structural hybridity between species must rest on other tests, these facts at least demonstrate that the cytological conditions for it can exist.

The occasional transmission of duplication gametes from translocations has been reported by a number of authors (e.g., BURNHAM 1934, 1948; LEWIS 1951; RHOADES 1933; SUTTON 1939). Recovery of deficiency-duplications has also been reported by BURNHAM (1932) in maize, SEARS (1939) in *Triticum vulgare*, and SMITH (1948) in *Triticum monococcum*. The deficiency-duplica-

tion described by SMITH is apparently analogous to the $2n + A - x$ class in the present study, though in his case the size difference between "A" and "x" is not so marked, both being rather short terminal segments.

So far as is known to the authors, however, no case has previously been reported in plants in which the array of unbalanced complexes approaches the theoretical possibilities as closely as in the present case. An explanation for the apparent rarity of such cases is not readily forthcoming. SMITH (1948) points out that deficiency-duplications ought to be fairly common in polyploid species, especially where deficiencies of whole chromosomes are known to be tolerated.

It may be pointed out that the extreme inequality of the interchanged arms, and the resulting effect on the location of chiasmata, render the present translocation unusually favorable for cytological detection of unbalanced complexes. It is not improbable that some such types have previously escaped detection. Because of its particularly convenient features, the 2B-1 translocation is the first to have been analyzed extensively in cotton. It is of course not certain or even probable that all translocations will yield the same array of unbalanced types. But it seems likely that if others can be found with the favorable features of 2B-1, some will prove to show similar behavior.

SMITH (1948) has discussed the possible uses of deficiency-duplication lines, and many other possibilities present themselves in special cases. The lines from 2B-1 are themselves useful. Perhaps more significant, however, are the implications they hold for future cytogenetic methods in cotton. The simplest models for studying linkage are those which make use of the monosomic method or its variants, since these are qualitative tests. While monosomics are now known to be transmissible in cotton in at least some cases, a promising method for their production has hitherto been lacking. Results of experiments with asynaptic plants have been disappointing. But translocations are rather easily induced by radiation, and the 2B-1 derivatives show that simple monosomics can be recovered from translocations. In practice, duplication-deficiency types may, however, prove both more easily recovered and more useful, since they are likely to be transmitted with greater regularity, and since some of them make it possible to determine concurrently the location of genes and the particular region of the chromosome concerned.

Aside from the possible contribution of 2B-1 and similar translocations to the cytogenetic exploration of *G. hirsutum* itself, certain of the derivatives present attractive possibilities of comparing the chromosomal and genetic anatomy of the allotetraploid species with that of their diploid relatives. Information of this sort should have considerable bearing on problems of transference of desirable characters from the diploid species into the cultivated allotetraploid cottons.

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CONTINUOUS SELECTION FOR EGG PRODUCTION IN POULTRY

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THE genetic bases underlying the changes in populations under artificial selection have been extensively explored both from the theoretical and the experimental approaches. The simplest situation in which selection is directed towards single traits, whose phenotypic variance may be regarded as the sum of the additively genetic and residual, or environmental, variances, does not appear to offer any serious problems at the outset of a selection program (vide the expositions in the texts of LUSH 1945; MATHER 1949; LERNER 1950).

However, even though the genetic situation might be adequately represented in this simple way at the start of a selection program, serious complexities may arise as the program is continued over many generations. One possibility, demonstrated to have occurred in a selection experiment for shank length in chickens (LERNER and DEMPSTER 1951) is that natural selection may come to exercise a strong effect countering that of artificial selection. Other disturbing influences may be anticipated on theoretical grounds, such as the development of a negative correlation between component characters, alterations in the phenotypic effects of given gene substitutions in response to altered genetic backgrounds, increases in the relative importance of overdominance and other forms of nonadditive gene interactions, the emergence of seriously detrimental characteristics, such as infertility, as concomitants of the characters subjected to conscious selection, and possibly a decreased environmental variance of the superior genotypes.

Because of these possibilities, and especially in view of the decreases in rate of gain assumed to have occurred after prolonged selection in domestic animals (see discussion by LUSH in CRAFT *et al.* 1951) studies of changes in heritability and other parameters of populations over many generations of selective breeding are necessary for an understanding of the problems involved in the formulation of efficient breeding plans for partially improved flocks or herds.

In the investigation referred to above (LERNER and DEMPSTER 1951) two populations were discussed, one under selection for increased shank length, and the other for a high production index (average first laying year hen-housed egg production). In neither case did there appear to be a marked reduction in genetic variance or in selection differential. Natural selection was shown to exert an important influence in a direction opposite to that of artificial selection in the shank-length selected flock, but no such influence was detected in the population selected for a high production index. The present discussion consists of a further analysis of the latter case, dealing with (1) the

correlation between viability and egg number, two components of the production index, (2) an analysis of the relationship between egg production and the reproductive fitness of the birds selected as parents of each generation, and (3) the changes in the heritability of the production index and its components over the period of selection.

Detailed information on the history of the population, the methods of selection and management used, and on other relevant data have been previously given by LERNER (1950) and will not be repeated here. Suffice it to say that the population studied consisted of a flock of Single Comb White Leghorns varying in number between about 400 and 700 pullets per year (with one exception when the number dropped to 260 birds) in which the primary cri-

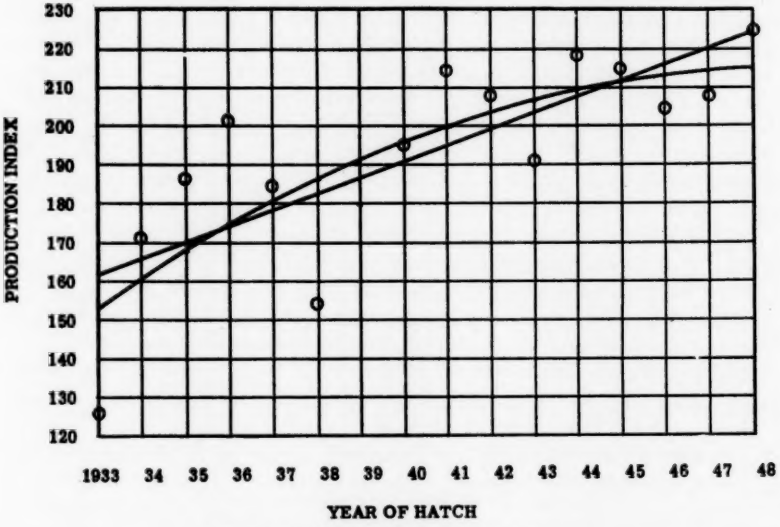

FIGURE 1.—The annual production index.

terion of selection was the production index of the birds themselves, their full-sister families, and, when available, their offspring. Starting with the level of approximately 120 eggs per year, the flock average rose in a somewhat irregular fashion, as shown by the circles in figure 1, to a level fluctuating about values of 200–210 with a high point of 225 eggs.

The linear regression, indicated by the straight line in the figure, has a slope of 4.10 eggs per year. The second degree regression, shown by the curved line, suggests that the rate of gain was quite high at the start of the selection program and has decreased over the years to a negligible value at the end of the period. However the coefficient of the second degree term, – .25, is not statistically significant ($P = .29$). The magnitude of the yearly fluctuations thus pre-

clude a definite conclusion as to whether the apparent decrease in rate of gain is a real one.

THE CORRELATION BETWEEN EGG NUMBER AND VIABILITY

The problem dealt with in this section refers to the question whether an attenuation of genetic progress in this flock could be accounted for by the induction of a negative correlation between the two components of the production index, egg number and viability, as suggested by LERNER (1950).

The material used for the study of this point consisted of six generations of the flock with some elimination of the small families. Three of the generations studied were selected from the early years of the experiment, and three from the later years when the apparent gains were much reduced.[1] The production indexes of the total population for three generations were:

1933	125.6	1941	213.5
1934	171.8	1942	208.7
1935	186.5	1943	190.9

Each generation consisted of birds surviving for the whole of the first laying year (through September 30 of the second year of life), which had complete production records, and of birds which died between housing (approximately five months of age) and the completion of the first laying year. The measures adopted as estimates of production and viability were respectively average daily production rate (APR) and age at death (AD).

The first of these for surviving birds simply represented the number of eggs laid from beginning of production through September 30 of the second year of life divided by the number of days from hatch to the above date. For birds dying in the course of their first laying year, the numerator of the ratio described above was an estimate of the number of eggs the bird would have laid had she survived the full year. The estimating equation (a different one for each month of death) was derived from the cumulative egg production records for different months of the laying year, previously reported by LERNER and CRUDEN (1948).

It may be assumed since heritability of first laying year viability is low (LUSH, LAMOREUX and HAZEL 1948; ROBERTSON and LERNER 1949), that in a great proportion of cases death is mediated by some nongenetic disposing cause, the genotype for viability being concerned, in part, with resistance to such variations or accidents of environment.[2] It is thus logical to attempt an estimate of the first year's production that a dying bird would have had if it had been subjected to an environment more favorable from the standpoint

[1] A limited number of computations reported here are based on the data tabulated by DR. H. M. EL-IBIARY.

[2] Most of the causes of death in this flock were classified as of non-specific origin (TAYLOR, LERNER and DEOME 1944). The only mortality of presumably infectious origin was that caused by leucosis, the heritability of resistance to which is under 10 percent (ROBERTSON and LERNER 1949).

of survival. However, many nongenetic influences tending to hasten death may be expected to affect rate of lay before death occurs. Ideally the impact of such effects on correlation between rate of lay and age at death would be separated by analysis of covariance between and within families. However, it is desirable from the standpoint of sampling error, and because some of the assumptions on which such separation rests may be only approximately true, to minimize as far as possible the influence of nongenetic variations simultaneously affecting egg number and age of death on the estimated production. Towards this end (and since the predisposing nongenetic causes of death are likely to produce their maximum effects on egg number in the last weeks of life), the estimating equations were applied to the number of eggs laid by nonsurvivors up to one month prior to their death.

The two measures then respectively represent (1) the real or estimated average daily production for the year of laying (APR), and (2) the age in months of the survivors on September 30 of their second year of life and of the nonsurvivors at the time of death.

Phenotypic and genetic correlations between APR and AD were computed far each of the generations and pooled estimates for the first three (1933–35) and the last three (1941–43) were derived. The phenotypic correlation for the first period (representing 1639 pullets) was found to be 0.19. The corresponding estimate for the second period (1466 pullets) was 0.09. The respective genetic correlations were 0.20 and 0.49.

If we take these figures at their face value, we would have to conclude that the genetic correlation is not only positive but has undergone a marked increase. The decrease in phenotypic correlation would probably indicate, in view of the increased genetic correlation, a still greater decrease in correlation due to nongenetic influences acting on rate of lay prior to one month before death. However, the sampling errors of the genetic correlations are considerably greater than those of the phenotypic correlations as is evident from the following table showing the values of the correlation coefficients for each generation separately.

Generation	Phenotypic	Genetic
1933	0.15	0.28
1934	0.21	−0.26
1935	0.20	0.73
1941	0.12	0.67
1942	0.05	0.98
1943	0.10	0.21

On the assumption that the variation between the three years of each period is largely due to sampling error, and that the error variance is the same for each period, a t test with 4 degrees of freedom can be applied for evaluating the significance of the pooled values and their differences. The phenotypic correlations, on this test, are significant for both the early and late periods ($P < .001$ and $P = .010$ respectively), and the decrease between periods is also significant ($P = .027$). The genotypic correlations on the other hand are not, although the value for the second period approaches significance ($P = .07$).

The magnitude of sampling variations therefore preclude reliable conclusions regarding the apparent increase in the genetic correlations although the value for the second period, at least, is very likely positive.

An attempt has been made to obtain information on the point of issue in a somewhat different way, namely on the basis of correlation estimates of the mean age of death in full sister families and mean production of the survivors in these families. The inter-sire component of this correlation, if significant, should give a reliable indication of the sign of the genetic correlation between AD and APR. The actual value might be somewhat attenuated; thus if there were a positive genetic correlation, a low mean genotype for survival in a sire family would tend to remove borderline birds from the class of survivors, increasing somewhat the mean level of the remainder. It can be shown that this tendency, in the event of normal distributions, could not reverse the sign of the correlation obtained. The intra-sire component of the computed correlation would be affected by maternal effects if these exist in any important degree.

The estimates of the components are shown in table 1. Neither the trends nor the differences from zero of the mean components, nor the totals are statistically significant. The results, while adding but little evidence to those previously obtained, are in agreement with them to the extent that both of the between-sires components are positive in sign.

TABLE 1

Correlations between mean age of death and the production of survivors in full sister families.

Year of hatch	1933	1934	1935	1933–35	1941	1942	1943	1941–43
Number of families	49	85	69	203	65	61	75	201
Between sires	.943	−.392	.597	.379	−.124	.255	.300	.174
Within sires	−.017	−.160	.013	−.058	.326	.184	−.101	.093
Total	.220	−.202*	.130	.033	.199	.201	−.014	.114

*Significantly different from zero at .05 level.

Some additional information on the point at issue may be obtained by comparing the within-sires regression of surviving daughters on dams with that of the mean actual production of all daughters on dams. The dams themselves are necessarily first laying year survivors, since no pullets were used as parents in the material studied. Hence twice the regression of surviving daughters should give an estimate of heritability of survivors' production. This estimate is expected to be negatively biased because of the use of family averages in selection, so that the latter is partly genotypic.

The heritability estimates thus obtained (see a later section of this article) are for 1933–1940, .418; and for 1941–1948, −.160.

While the latter figure is not significantly different from zero, it seems that the heritability of survivors production estimated in this particular fashion has decreased in the latter stages of the selection program. On the other hand, twice the intra-sire regression of the mean *production index* of all daughters

TABLE 2

Correlations between reproductive characters of two-year-old dams mated for the first time. Columns (f) and (h) are based on the first four weekly hatches of each breeding season.

Year of hatch	Number of hens	Mean first year production (x)	Mean number of fertile eggs (f)	Mean number of chicks hatched (h)	r_{fx}	r_{hf}	r_{hx}	$r_{hx.f}$	b_{fx}	b_{hf}	b_{hx}	$b_{hx.f}$
1934	43	244.0	17.6	13.5	−.171	−.895	−.145	.018	−.054	.785	−.040	.002
1935	48	249.2	13.0	10.2	.258	.937	.263	.063	.087	.810	.077	.007
1936	61	262.5	13.5	9.8	.274	.879	.171	−.152	.093	.773	.051	−.023
1937	65	274.7	15.7	12.3	−.034	.942	.048	.239	−.010	.893	.013	.021
1938	52	282.5	15.2	12.2	−.113	.888	−.166	−.144	−.037	.814	−.050	−.020
1939	48	277.7	15.5	10.9	−.001	.906	.034	.083	−.001	.765	.011	.011
1940	38	276.3	12.1	8.3	−.236	.936	−.187	.099	−.086	.808	−.059	.011
1941	68	279.0	15.8	12.2	.029	.915	.032	.014	.007	.824	.007	.001
1942	49	284.9	14.2	9.7	.075	.936	.084	.039	.032	.738	.028	.004
1943	90	289.4	15.1	11.7	.289	.862	.174	−.155	.085	.782	.046	−.022
1944	52	283.0	15.2	10.8	.011	.850	−.044	−.101	.003	.779	−.010	−.012
1945	67	268.4	15.0	11.6	−.080	.885	−.046	.053	−.024	.846	−.013	.007
1946	74	283.9	16.1	12.6	.118	.892	.121	.035	.039	.856	.038	.005
1947	68	294.9	14.2	11.4	−.011	.929	−.083	−.197	−.005	.880	−.033	−.029
1948	77	282.1	15.5	11.8	.164	.849	.020	−.228	.056	.878	.007	−.043
1949	89	291.9	16.1	12.7	.191	.879	.054	−.244	.052	.808	.013	−.030
1934–41	423	269.1	14.9	11.3	.013	.912	.018	−.014	.004	.811	.005	.002
1942–49	566	285.3	15.3	11.7	.115	.884	.050	−.112	.036	.819	.014	−.015
1934–49	989	278.3	15.1	11.5	.068	.896	.035	−.058	.021	.815	.010	−.007

on dams has increased as shown by the comparisons of the values for 1933–1940, .368; and for 1941–1948, .688.

The latter regression involves the genetic correlation between the production of survivors and viability, while the regression of surviving daughters on dams does not. Hence, the difference in the trends of these two regressions suggest, not only a positive genetic correlation in the later years, but a considerable change in the plus direction between the early and later years. However, possible alternative explanations should be considered. An increase in the regression of all daughters on dams could result from an increase in the genetic correlation between production of survivors and production of nonsurvivors. Since there has been a great increase in the number of eggs laid by nonsurvivors, an increase in this correlation is not unlikely although it is difficult to explain more than a part of the relative trends of the two kinds of regressions on this basis. Such a correlation would tend to increase rather than decrease the effectiveness of selection. Another, and more likely, explanation is sampling error.

In any case, all of the three methods considered in this section suggest an absence of a negative, if not necessarily the presence and increase of a positive, genetic correlation between genotype for survival and genotype for production of birds if they survive. It therefore seems apparent that the induction of a negative correlation between the components of the production index suggested by LERNER (1950) cannot be invoked in explanation of the attenuation of gains observed in this flock.

EGG PRODUCTION AND HATCHABILITY

In the previously cited report (LERNER and DEMPSTER 1951) on the analysis of reproductive behavior of the females selected to be parents of each succeeding generation of the flock under study, no indication of a negative relation between egg number and reproductive fitness was found. This nevertheless does not preclude the possibility that individual components of fitness may be adversely affected by high egg production. Accordingly an analysis of correlation between egg number in the first laying year, the number of fertile eggs produced in the course of a standard four-week hatching season in the spring of the birds' second year of life, and the number of chicks produced was undertaken.

Only two-year-old dams previously unmated were included in this analysis, and the correlations and regressions were computed on the intra-sire basis. The complete analysis appears in table 2, the last three lines summarizing the information with respect to the first part of the selection period, the second part, and the whole. The figures appearing in the table make it abundantly clear that no important differences in hatchability or number of chicks produced exist between the periods of high and low genetic gain.

The absence of correlation between egg number and hatchability in this case is contrary to many earlier studies, in particular that of LERNER and GUNNS (1952) on the same flock. The discrepancy is, however, not real, since in the

latter case the whole range of egg production records was represented, while in the present table 2 a sample of only the extreme right hand tail of the distribution is included.

It seems significant that contrary to the results obtained by LERNER and DEMPSTER (1951) in their shank-length selected population no decrease in reproductive fitness has occurred in this population in the course of selection. In other words, here improvement in the production index was obtained at no cost in fitness of the population. The general notions of genetic homeostasis developed by us previously do not thus appear to explain the attenuation of gains, a fact already brought out earlier (LERNER and DEMPSTER 1951), and further confirmed by the current analysis.

TRENDS IN HERITABILITY

The next source of gain attenuation which is worthy of examination lies in the possible changes in total heritability or in its additive component. It is not impossible that effective genetic variance has decreased in the course of selection, both because of the selection itself and because of increased homozygosity from inbreeding occasioned by the small population size. Neither of these two causes is likely to be great in effect. The former (selection) in a multi-genic situation over a period of only a few generations cannot be expected to decrease variance to any great extent unless some of the alleles subject to the discriminatory action of selection have quite large differential effects; under certain circumstances selection may actually contribute to an increase in genetic variance. The latter (inbreeding) has proceeded at the rate of approximately one percent per year as computed from increases in the inbreeding coefficients. The real increase may have been less if heterozygotes were favored by either natural or artificial selection.

A third possible cause, however, must not be overlooked, namely the effect of a different average genetic background (as evidenced by increases in the production index) on given gene substitutions. The direction of the effect produced by this factor cannot be known a priori: on the one hand a decrease in the variance could be expected due to a possible approach of the average production to whatever physiological limits may exist; on the other, an increase may obtain because the heritability of the production of survivors is relatively high and the proportion of survivors in the flock increased. In addition to quantitative changes in heritability, changes in specific gene frequencies and in over-all genetic background might alter the proportion of the variance that is additive.

The heritability of the production index may be thought of as a function of the heritability of survivors' production, the heritability of survival, and heritability of nonsurvivors' production. We will first consider the heritability of survivors' production and its relationship to that of the production index. We will next consider changes in the other two components of the production index, and their possible contribution to heritability of the index. Yearly estimates of heritability of survivors' production and of all birds, are given in

TABLE 3

Estimates of heritability of survivors' production.

Year of hatch	Number of pullets	Heritability estimates			Intra-sire daughter-dam regression	
		From dam components	From sire components	Joint	Number of dams	Regression
1933	215	1.152	.176	.664	....	....
1934	356	.061	.284	.172	43	.130
1935	279	.385	.440	.413	30	.406
1936	219	−.029	.823	.397	35	.153
1937	286	.185	.163	.174	49	.347
1938	241	.333	.356	.345	34	.453
1939	234	.147	.197	.172	33	.027
1940	161	−.093	.381	.144	23	−.048
1941	256	.168	.166	.167	46	−.347
1942	240	.605	.208	.406	28	.114
1943	479	.177	.102	.139	72	−.117
1944	171	−.046	.222	.088	30	.167
1945	281	−.290	−.025	−.158	49	.188
1946	391	.179	.103	.141	53	−.157
1947	312	.276	.110	.193	44	.113
1948	436	.393	.372	.382	56	−.216

tables 3 and 4 respectively, while table 5 gives pooled values for the early and late periods of selection as well as for the total. The discrepancy between the numbers of birds used in the analysis and those in the previous report of LERNER and DEMPSTER (1951) is accounted for by the elimination of small families. Furthermore, in the computation of intra-sire daughter-dam regressions only two-year-old dams were used. Thus in each generation all the dams

TABLE 4

Estimates of heritability of the production index.

Year of hatch	Number of pullets	Heritability estimates			Intra-sire daughter-dam regression	
		From dam components	From sire components	Joint	Number of dams	Regression
1933	509	.305	.128	.216	....	....
1934	685	.137	−.004	.066	47	.191
1935	470	.199	.171	.185	35	.814
1936	369	−.009	.178	.085	47	−.014
1937	567	.085	−.060	.013	56	.471
1938	585	.428	.010	.219	43	.199
1939	350	.256	.073	.165	38	−.163
1940	258	.889	−.172	.359	26	−.537
1941	465	.149	.140	.144	57	.536
1942	344	.304	.253	.280	33	.276
1943	638	.178	.181	.179	72	−.071
1944	244	−.109	.224	.057	38	.474
1945	441	.155	.069	.112	56	.605
1946	474	.204	−.017	.093	55	.055
1947	458	.057	.271	.164	52	.210
1948	548	.443	.175	.309	56	−.416

were contemporary with each other. In tables 3 and 4 the actual regressions are given. In table 5 these have been converted into heritability estimates.

On the assumption that there is a linear relationship between heritability and year and that the deviations from the regression of one on the other are random and normally distributed, estimates of heritability trends were made for the survivors' production and the production index. The regressions and estimates of heritability from them, made for the first year, last year, and the center of the regression line are given in table 6.

The estimates of heritability of survivors' production from the dam and the sire components are very similar both for the early and late years. This is to be expected if the genetic variance is additive. Nonadditive genetic variance would usually tend to increase the dam-component estimates relative to those from the sire components. The regression coefficient of the joint estimate on year is negative and almost significant at the five percent level. The coefficients

TABLE 5

Pooled estimates of heritability

	Production index			Survivors' production		
	1933–1940	1941–1948	1933–1948	1933–1940	1941–1948	1933–1948
Degrees of freedom	3785	3604	7389	1983	2558	4541
Pullets per dam	6.74	5.97	6.34	4.29	4.67	4.50
Pullets per sire	46.83	43.00	44.88	25.53	30.55	28.13
Heritability estimates:						
From sire component	.252	.198	.225	.259	.201	.213
From dam component	.046	.155	.099	.335	.160	.217
Joint	.149	.177	.162	.297	.181	.215
From pullet-dam regression*	.368	.688	.568	.418	−.160	.092
Phenotypic variance	7464	7369	7418	2163	3305	2806

*These figures are subject to a much greater sampling error than the others (the degrees of freedom listed do not apply to them).

for the separate components, although not themselves significantly different from zero, are both negative and similar in magnitude to that of the joint estimate.

The joint estimate at the center period (assuming linear regression) is .24 ± .04, a moderately high value very significantly different from zero. The indications then are of a decrease from a heritability around .38 at the beginning of the period to .10 at the end, although little reliance can be placed on the estimated extent of the decrease.

The heritability of the production index shows an entirely different picture. The regression estimate, based on the sire component, is much lower than that based on the dam component at the start of the period (.047 vs. .236) and is only a little lower at the end of the period (.156 vs. .222). The difference at the center period is nearly significant (P less than .1). The regression is negligible in the case of the dam component (slightly negative) but positive and much larger in the case of the sire component, although even here statistical significance is not reached on the hypothesis of linear regression ($P = .29$).

TABLE 6

Regressions of measures of heritability on year of hatch and heritability estimates based on these regressions.

Character	Component	Regression coefficient	t	P	h^2 estimates		
					1933	1940–41	1948
Survivors' production	Dam	−.0205 ± .0176	1.17	.26	.379 ± .155	.225 ± .081	.071 ± .155
	Sire	−.0172 ± .0100	1.73	.11	.384 ± .088	.255 ± .046	.126 ± .088
	Joint	−.0188 ± .0092	2.05	.06	.382 ± .081	.240 ± .042	.098 ± .081
Production index	Dam	−.0009 ± .0129	0.07	> .9	.236 ± .114	.229 ± .060	.222 ± .114
	Sire	+.0072 ± .0066	1.09	.29	.047 ± .058	.101 ± .031	.156 ± .058
	Joint	+.0031 ± .0051	0.61	.54	.142 ± .045	.165 ± .024	.189 ± .045

If the indicated trends are real the heritability of the production index, the character most emphasized in selection, has changed little in magnitude but starting with a large nonadditive fraction has become more, and perhaps largely, additive as selection progressed. By contrast, the heritability of survivors' production appears to be largely additive throughout and to be gradually diminished as a result of inbreeding and selection. These relationships are not established with any high degree of significance but are the most likely conclusions to be drawn from the data. While little reliance can be placed on the heritability estimates based on within-sires dam-daughter regressions (table 5), the relative trends (i.e., a decrease for the heritability of survivors' production and an increase for that of the production index) agree with those based on variance components.

The heritability of viability in this flock has been investigated by ROBERTSON and LERNER (1949). The joint pooled estimate (based on dam and sire components for the first twelve years of the selection program) is .089. Two standard errors were computed, one based on within years' analyses, and the other on the variation of the estimates from year to year. The two estimated errors are .28 and .24 respectively. In the present case, it is of particular interest to know whether the heritability has changed over the period of selection. A priori, a decrease might be expected, particularly in view of the decreased mortality and consequent greater departure of the dichotomy from the 50% point. Taking the estimates for the individual years, and assuming linear regression, the following results are obtained:

Regression	h^2 estimates	
$b = .011 \pm .007$	Beginning of period	$.026 \pm .062$
$t = 1.6$ $P_{10\ d.f.} = .14$	Center of period	$.084 \pm .023$
	End of period	$.142 \pm .062$

The assumption of regression and equality of weighting for the different years accounts for the slight differences in the central estimate and its standard error as compared with the pooled estimate and error cited above. The data suggest that the heritability of survival has actually increased during the period of selection, although the probability that the observed regression could have occurred by chance is no lower than .14. If this increase is genuine it would contribute toward the apparent increase in the heritability of the production index, in the face of a decrease in that of the production of survivors.

One result of selection has been a decrease in mortality. The average proportions of birds dying for the two three-year periods mentioned previously are .35 and .22, the decrease being highly significant ($t = 4.77$; d.f. = 4, $P < .01$). If q is the proportion of birds dying and d the difference between the mean numbers of eggs laid by surviving and dying birds, the contribution of mortality to the total variance of the production index can be expressed as $q(1-q)d^2$. These contributions for the two periods, expressed as a percentage of total variance, are 50 percent and 31 percent respectively. The reduction can help to account for the maintenance or increase of the original level of

heritability of the production index because the heritability of mortality is lower than that of the production of survivors.

The heritability of the number of eggs laid by nonsurvivors, mentioned above, may appear to be something of a misnomer inasmuch as the dead birds cannot leave offspring. However the proportion of variance of this character attributable to genetic causes has a direct bearing on gains achieved by family selection. It is possible to compute this heritability by variance analysis, just as heritability has been computed for the survivors' production. In either case the heritability would depend in part on the values of genetic and nongenetic correlations between rate of lay and survival. Thus a high nongenetic correlation between these two variables and a low genetic correlation could decrease the nongenetic variance in number of eggs laid within each of the two classes (survivors and nonsurvivors) without decreasing the genetic variance; this would result in higher estimates of heritability. If the mortality were around 50 percent in the early years, such a positive bias in heritability estimates within the two classes would be relatively small; as mortality decreased over the years the bias would be gradually lost in the increasing class of survivors

TABLE 7

Heritability estimates of number of eggs laid by non-survivors

Year of hatch	From dam component	From sire component	Joint estimate	Year of hatch	From dam component	From sire component	Joint estimate
1933	−.009	.088	.040	1941	.24	.11	.17
1934	.43	−.11	.16	1942	.82	.14	.48
1935	.85	.23	.54	1943	−.11	.31	.10
Pooled	.44	.05	.24	Pooled	.21	.19	.20

but considerably augmented in the decreasing class of nonsurvivors. Changes of this nature due to the shifting of individuals from one class to another would presumably have no direct effect on gains from selection, although the indirect effects previously discussed might be large.

Estimates of the heritabilities of nonsurvivors are shown in table 7. In view of the magnitude of their variation, none of the changes can be considered significant, although the difference from zero of the mean joint estimate (.22) is significant ($P = .035$). The joint estimates for the two periods are nearly the same, but the estimate from the sire component has increased and that from the dam component has decreased. This would suggest a rise in the proportion of genetic variance that is additive, which is in line with the apparent trend of the heritability of the production index.

DISCUSSION AND CONCLUSIONS

Detailed analyses undertaken with the aim of determining the reasons for the apparent decline in response to selection unexpectedly lend support to the view that a real decline may not have occurred.

It has been previously shown that the reductions in both family and individual selection differentials have been very moderate amounting to about 17 percent for the former and 9 percent for the latter in the last half, as compared to the first half, of the selection program (LERNER and DEMPSTER 1951).

One obstacle to improvement, which might be expected on theoretical grounds, would be the development of a negative genetic correlation between two important components of the production index, namely ability to survive the first year's production and rate of lay. Three tests made of this point indicate that the genetic correlation between these components is probably positive. Two of them also suggest that the genetic correlation may have actually increased. These results cannot be considered conclusive, but at least no evidence was uncovered for a decreasing or negative genetic correlation between the variables involved.

An analysis of the relationship between reproductive fitness and first year production, extending one previously made (LERNER and DEMPSTER 1951), confirms the earlier conclusion that natural selection is not operating counter to artificial selection, and that there is no evidence indicating any change in the action of natural selection during the period covered.

The trend of heritability of the production index as estimated from the combined variance components due to sires and dams shows a small increase. This increase is due to the changes in the estimates from the sire component, the dam component showing a negligible decrease. Taken at face value, these figures would indicate not only that the heritability has not decreased (and may in fact have risen), but that it has become more additive, since nonadditive genetic variance is expected to produce a much larger maternal than paternal variance component. None of these trends, however, is statistically significant so they can be accepted only as contributory evidence.

The heritability of survivors' production appears to have decreased markedly. The significance level of the regression coefficient, however, is only .06. The estimates both of heritability levels and of trends, based on sire and dam components separately, are very similar. This suggests that the genetic variance of survivors' production is largely additive in nature. If so, then the nonadditive component of the genetic variance of the production index must be related to the contributions from mortality and, possibly, from the variance of the production of birds dying during the first year of production.

It seems difficult to account for an increase in heritability of mortality on purely genetic grounds; if such an increase has actually occurred, as the regression coefficient suggests, it may be related to changes in the nature of environmental conditions usually responsible for deaths during the first year. Although statistical significance for an increase in heritability of viability has not been reached ($P = .14$), there is nothing in the data indicating a decrease during the period of selection. Any change would not be expected to affect directly the heritability of the production of survivors, but an increase would tend to raise the heritability of the production index, and hence is consistent with the indicated trends of both of these heritabilities. Decreased mortality

itself, as discussed above, should greatly reduce the contribution of mortality to total variance, as well as the contribution of variation in egg number among nonsurvivors, and thus tend to equalize the heritabilities of the production index and of survivors, in accordance with the observed trends.

Little can be concluded concerning the trend of estimated heritabilities of nonsurvivors, except that the apparent increase in additivity is also consonant with the trends of production-index and survivors'-production heritabilities. The same can be said concerning the indications of a positive and perhaps increasing genetic correlation between rate of lay and age at death.

It thus seems that although the rate of gain may appear to have decreased, the evidence from the trend of the production indexes for successive years is rather slight and receives little corroboration from the detailed analyses. The only supporting subsidiary evidence is the probable decrease in the heritability of survivors' production. This decrease, however, seems to be compatible with an increase in the heritability of the production index itself. Evidence against any marked decrease in rate of progress includes the lack of any marked reduction in selection differential, the absence of an adverse effect of natural selection, an apparently positive if not increasing genetic correlation between survival and rate of lay, an apparent increase and greater additivity of the heritability of the production index, a possible increase in the heritability of mortality, and a decrease in mortality together with a higher average heritability of survivors' production as compared to that of viability. Although none of these lines of evidence are decisive when considered alone, taken together they point to the improbability of any close approach to actual cessation of gains and suggest that response to selection may not be very different at the present stage of the breeding program than during the first few years of improvement. Any tentative conclusion to this effect should be viewed in terms of the following conditions of this experiment: selection for a character that constitutes an important component of reproductive fitness, fairly strong selection intensities (averaging more than one standard deviation for both family and individual selection), a relatively low heritability (averaging approximately .10 for the production index, as indicated by half sister correlations which should approximate the additive rather than the total heritability value), somewhat more than eight average generations of selection, and a computed average increase of homozygosity, based on inbreeding coefficients, of about 15 percent. LUSH (CRAFT *et al.* 1951) asks whether the dilemma of decreasing gains from selection in the presence of nearly the same amounts of genetic variability and the same type of selection pressures at the beginning as at later stages of a breeding program really exists. As far as the present data are concerned, a negative answer to this question appears not improbable.

ADDENDUM

A number of changes in mating system and management have been introduced in the years following those analyzed here. In addition the generations hatched in 1949 and 1950 were subject to epidemics of respiratory diseases to

which the flock has not hitherto been exposed. The consequent drop in production was so severe as to preclude effective selection for egg number in these generations. The 1951 generation, completing its production year on September 30, 1952, however, bears out the favorable prognosis for further gains, which could be made on basis of this analysis. The production index of the part of the flock under management identical with the one described here, consisting of 324 birds, reached 231.3 eggs, a value exceeding the previous high point in figure 1.

SUMMARY

1. The production index (average number of eggs laid by all birds, survivors and non-survivors, during the first year of lay) in the University of California production flock appears on the surface to have improved at a decreasing rate during sixteen years of selection. However, the second degree regression coefficient is not statistically significant.

2. No adverse effect of natural selection on the gains realized is in evidence.

3. There is no indication of the development of a negative genetic correlation between rate of lay and ability to survive the first year of egg laying.

4. The heritability of the production index seems to have increased and become more additive, although this cannot be established with statistical significance.

5. Although the heritability of the production of birds surviving the first laying year has probably decreased, the evidence on other components of the production index and their inter-relationships is consistent with a possible increase in the heritability of the latter.

6. It appears *improbable* that response to selection has ceased and *possible* that gains in relation to selection intensity may still be obtained at approximately the rate characteristic of the early years of the improvement program.

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SULFUR-35 AS A MUTAGENIC AGENT IN NEUROSPORA[1]

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WE have undertaken to determine whether radioactive sulfur (S^{35}) can induce mutations, not only by its radiation, but also by its concurrent transmutation. We have found that under some conditions S^{35} is an extremely powerful mutagen on *Neurospora crassa*. From the determination of the amount of mutation induced when varying amounts of carrier sulfur are fed with constant amounts of radioactive sulfur, it appears that a large number of the mutations arise from the transmutation of S^{35} into chlorine-35 apart from those produced by the concurrent radiation.

BARRON and DICKMAN (1949) find that the activities of several sulfhydryl enzymes are lost by the destruction of the – SH group. The apparent similarity of genes and enzymes suggested that if one could destroy the sulfhydryl groups on the genes controlling the production of sulfhydryl containing enzymes, then this should be observable as a mutation. Such ideas have been tested by CLARK *et al.* (1950) by attempting to attack the – SH group with specific chemicals. While this work did show that mutations can be produced by such treatment, the yield was not high. Any conclusions based on such experiments are always shadowed by the question whether the chemical agent can actually enter and be in position to attack the gene without destroying the cell.

Since there is no problem of penetration by the radioisotope, the primary problem in our work has been to differentiate between the two mutagenic potentials within the radioactive atom. First, the weak beta particle which is emitted when this isotope disintegrates, is known to be a nonspecific mutagen. Second, when the beta particle has been emitted, this atom is no longer a sulfur atom but rather a chlorine atom. If a gene contains a sulfur atom essential to the functioning of the gene, the conversion of the sulfur to chlorine surely would modify the properties of this group and might result in a mutation. This should be true whether the converted atom is ejected from the molecule by its recoil energy or stays in the molecule as a chlorine atom.

Distinguishing the effects of one from the other of these potentials can best be done by keeping the radiation intensity constant while the transmutation is varied. This might be accomplished in either of two ways. (1) Grow the test organism on a metabolizable source of S^{35} and compare the mutation rate

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[3] We wish to express our appreciation for the constructive criticism given by DR. ALAN MACEWAN.

produced (by both radiation and transmutation effects) with that found in a similar culture grown on a nonmetabolizable source of S^{35} (only radiation could cause mutation). (2) Grow the test organism on a constant amount of metabolizable S^{35} but different amounts of nonradioactive sulfur (S^{32}) and compare cultures in which the ratio of S^{35} to S^{32} varied (radiation constant, varying probability of transmutation). The second of these methods was chosen as the one more likely to answer our questions.

Neurospora crassa was selected for use because of its simple nutritional requirements and well known genetics. This mold is able to utilize sulfate which is a form of sulfur readily obtainable from Oak Ridge in carrier-free form.

Before starting this work, it was necessary to determine the relationship between the amount of sulfur added to the medium and the amount of growth

TABLE 1

Growth on varying sulfur concentrations (weight in milligrams of dried mycelium after 3 days growth at 25.5° C on liquid medium).

Micromoles of added sulfur per ml	Run I	Run II	Run III
6	88.7		
4	84.7		
3	88.4		
2	83.7	69.9	
1.6	86.5	78.9	
1.2	89.9	80.5	
1.0			109
0.8	88.8	78.3	
0.4	83.3	79.3	
0.2	67.7	68.7	
0.1	47.4	50.0	52.5
0.05	33.3	33.2	
0.01			9.2
0.001			4.1
0.0001			3.1
0.0	4.0	4.0	3.0

obtained. Table 1 gives the results of an experiment in which a wild strain of the mold (1A) was grown in 125-ml Erlenmeyer flasks containing 15 ml of medium. The salts in this minimal medium were modified from the usual Fries-3 by adding varying ratios of $MgCl_2$ and $MgSO_4$ so that the molarity of the Mg^{++} was constant while the sulfur varied. It is apparent from these data that, while the mold needs at least 0.0004 molar sulfur in order to grow at the maximum rate, it is able to grow at extremely low levels of sulfur. Some growth is obtained even when no sulfur is added to a " sulfur-free " medium.

An initial experiment was set up to determine whether significant amounts of mutation could be recovered when S^{35} was the mutagen. Since it was found that some growth was obtained even when no sulfur was added, we decided to add only 1.1×10^{-10} moles (160 microcuries) of S^{35} to one ml of solid growth medium. This represented a compromise between the undesirability

of a high radiation intensity when more radioactive sulfur is added, and the necessity of keeping as high a specific activity as possible. In this particular experiment 0.1% yeast extract and 0.1% bacto-peptone were also added. Later it was realized that with this was added an unknown amount of S^{32}. The medium was sterilized and inoculated with a microconidial strain [4] of Neurospora and grown at 25°C.

After 26 days the conidia were removed, filtered through a sterile cotton plug to remove mycelial fragments, and plated on a complete medium [5] containing 0.1% sorbose (TATUM, BARRATT and CUTTER 1949). On this medium compact colonies grow from single conidia each containing a single nucleus. These colonies were picked and placed in small tubes containing complete medium. Testing by the methods of BEADLE and TATUM (1945), 5% of the isolates were biochemical mutants.[6] Compared with the rate of mutation obtained even with large X-ray dosages this recovery was high and thus very promising.

TABLE 2

Growth substrate (25-ml Erlenmeyer flasks each containing one ml of "sulfur-free" Fries-3 agar medium with additions as indicated).

Flask number	1	2	3	4	5	6	7
S^{35} as sulfate added (1.4×10^{-10} moles/0.2 mc)	0.2 mc	0.2 mc	0.2 mc	0.2 mc	2.0 mc	0.2 mc	0.0
Moles of S^{32} added as sulfate	0	1.3×10^{-9}	1.4×10^{-8}	1.4×10^{-7}	0	0	1.4×10^{-10}
Days exposure to isotope before plating	18	18	18	18	18	6	6

A series of flasks was then made up so that the same amount of S^{35} was diluted with varying S^{32}. In this way the total radiation was the same in all flasks and only the specific activity varied from flask to flask. The medium was made up as shown in table 2. The total sulfur added (a maximum of 1.4×10^{-7} moles per flask) was insufficient for optimal growth (table 1). The flasks were each inoculated with one drop of a dilute suspension of microconidia in distilled water. Most of the cultures were grown for 18 days. At the end of the growth period a portion of the agar and mold was removed from each culture

[4] Strain 8743-21 (13-7)a. Characteristics and history of this strain are given by BARRATT and GARNJOBST 1949.

[5] Fries-3 salt solution with 0.25% yeast extract, 0.25% peptone, 0,025% enzyme hydrolyzed casein, vitamin solution, 1% glycerin, and 5 gamma of biotin per liter.

[6] This mutation rate is calculated on the basis of the number of strains isolated which are clearly identifiable both in the initial minimal test and the succeeding test on four group supplements. It thus includes some strains whose requirements have not been completely identified. This will be the basis for the mutation rates given throughout this paper.

with a sterile microspatula. Each of these was then placed in a test tube of sterile liquid medium and the flask from which it was withdrawn was refrigerated. The conidia were removed, plated, and the colonies were isolated and tested as before for the presence of biochemical mutations. The isolations were done under a dissecting microscope so that none of the less well developed colonies would be missed. All discrete colonies were picked from each plate. Table 3 shows the relative percentages of biochemical mutants recovered from the flasks.

While the data derived from flasks 5, 6, and 7 are of considerable interest and supplement the other results, those derived from flasks 1, 2, 3, and 4 will serve as the body of data used in the discussion. These four flasks with identical amounts of radioisotope were exposed for the same length of time yet the mutation rate drops successively from flask 1 to 4. It is apparent from table 2 that the specific activity of the radioactive sulfur also drops successively in these same flasks. The flask in which the specific activity of the radioactive

TABLE 3

Numbers of biochemical mutants recovered.

Flask	Number of isolates	Amino acid mutants	Vitamin mutants	Yeast extract and peptone mutants	Simple carbon source mutants	Questionable mutants	Total biochemical mutants	Percent biochemical mutants
1	1208	92	27	29	7	28	183	15.2%
2	422	32	13	7	1	1	54	12.8%
3	942	46	21	15	4	14	100	10.6%
4	947	31	8	8	2	9	58	6.1%
5	(No growth)							
6	1054	47	15	16	3	7	88	8.3%
7	335						0	0.0%

sulfur was the greatest (No. 1) gave the highest rate while that with the lowest specific activity (No. 4) gave the lowest rate.

The difference in rate could be due to either of two things. First, it could be caused by a greater percentage uptake of sulfur and thus more radiation of the organism in those flasks containing less total sulfur. Second, it could be caused by the difference in the specific activity of the sulfur that is present in the four flasks, with a decrease of specific activity causing a lessening of the probability that a transmutation will cause a mutation. It is apparent that the choice between these two alternatives can be made only if the radiation intensity in the organism as well as in the medium can be determined.

To determine the relative amount of radiation administered to the organism the conidia were sampled to determine their content of radioactive sulfur. To do this, another square of agar and mold was removed from the same flasks. The conidia were suspended in 1 ml of water and filtered through cotton. After filtering, the conidia suspension was centrifuged and the supernatant decanted. The spores were then resuspended in water, centrifuged, decanted, etc., through three washes. After the spores had been centrifuged from the last

wash 0.1-ml samples of the spore-free supernatant were plated on aluminum planchets and dried. The centrifuge tubes containing the spores were then shaken to suspend the spores and a 0.1-ml sample of each suspension also plated and dried. The activities of these plates were counted under a thin end-window G.M. tube. Spore counts of the same suspensions were made with a haemocytometer. The results are collected in table 4. When the amount of radioactivity per conidium was calculated it appeared that approximately the same amount of radioactive sulfur was present in the conidia of all flasks except number four, where there was a noticeable drop in the amount of activity per conidium.

It is apparent that each conidium in flasks 1, 2, and 3 contains approximately the same amount of S^{35}. Assuming that there is no strong selectivity for the radioisotope over the stable one, the total amount of sulfur per conidium must vary between flasks since 10 parts of carrier sulfur were added to flask 2 and 100 parts to flask 3. If the differences in mutation rate were to be explained as a consequence of differences in specific activity of sulfur and the resulting difference in the probability of the transmutation of a S atom pro-

TABLE 4

Sulfur-35 content of the conidia

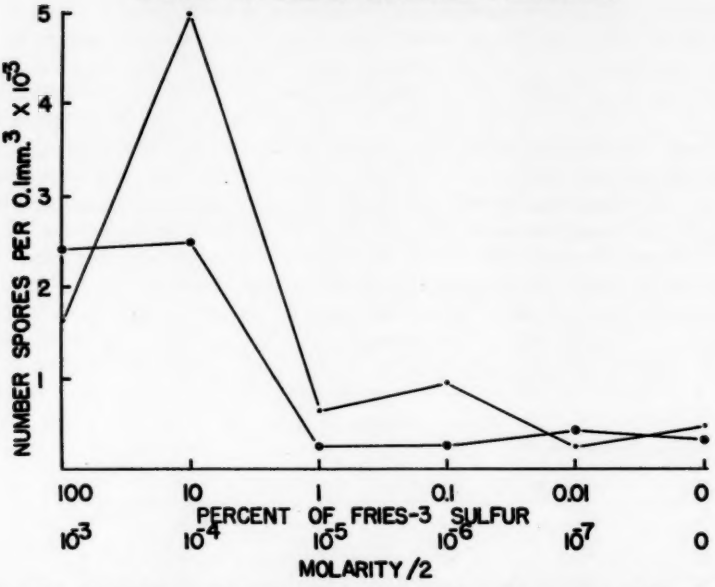
Flask	C.P.M. 0.1 ml supernatant (C)	C.P.M. 0.1 ml suspension (S)	(S − C)	Number spores per 0.1 ml × 10^{-3}	Counts per spore
1	656	6101 ± 57	5445	68 ± 9	0.08
2	765	8757 ± 21	7992	113 ± 16	0.07
3	1150	11285 ± 133	11135	141 ± 12	0.08
4	1334	18599 ± 53	17265	455 ± 46	0.04

ducing a mutation, it would be expected that the transmutation induced mutation rate would change in ten-fold increments. This, however, was not observed. There are two things which could account for this difference between observed and expected values. One is the fact that contaminating sulfur from the inoculating conidia and from the agar and inorganic salts must have been initially present. From table 1 it appears that even when no sulfur is added to the sulfur-free medium some growth is still obtained, thus, there must be some sulfur present initially even in the so-called " sulfur-free " medium. Also, it was found that the number of conidia produced by a culture varies with the amount of sulfur added (figure 1). With a slight decrease of sulfur below that present in ordinary Fries medium the number of conidia increases but as the concentration of sulfur in the medium continues to decrease, the number of conidia produced by the mold then decreases to a limiting lower value. Although we have not been able to obtain close consistency, this lower limit is reached when the added sulfur is between 10^{-5} and 10^{-6} molar. This approximates the figure obtained from table 1.

Furthermore, by using the data in table 3, it is possible to calculate the amount of sulfur initially present in the medium as well as the amount of

FIGURE 1

EFFECT OF SULFUR ON CONIDIA PRODUCTION



mutation induced by radiation and the amount induced by transmutation. This is done by assuming that the amount of mutation induced by radiation is the same in each flask and that the variation in the total observed mutation rate is brought about solely by the change in specific activity of the S^{35}. The frequency of mutation due to transmutation in flask 1 (M_{t1}) is to the frequency in flask 2 (M_{t2}) as the ratio of sulfur disintegrated (S_{dis}) to total sulfur in flask 1 is to the corresponding ratio in flask 2:

1. $$\frac{M_{t1}}{M_{t2}} = \frac{S_{dis}/S_{total-1}}{S_{dis}/S_{total-2}} = \frac{S_{total-2}}{S_{total-1}} = \frac{S_{35} + S_{c2} + S_x}{S_{35} + S_{c1} + S_x}$$

when the S_{total} is made up of the S^{35} added (S_{35}), the S^{32} added as carrier (S_c), and the sulfur that already was present in the medium (S_x). Since the frequency of mutation from radiation (M_r) should be the same in all flasks, it follows that:

$$M_{t1} = M_{total-1} - M_r = 15.2 - M_r$$
$$M_{t2} = 12.8 - M_r$$
$$M_{t3} = 10.6 - M_r$$

The values for added sulfur are known to be the following:

$$S_{35} = 8.0 \times 10^{13} \text{ atoms} = 1.33 \times 10^{-10} \text{ moles}$$
$$S_{c1} = 0$$

$S_{c2} = 0.78 \times 10^{15}$ atoms $= 1.29 \times 10^{-9}$ moles
$S_{c3} = 8.4 \times 10^{15}$ atoms $= 1.38 \times 10^{-8}$ moles

Using these values for sulfur and the values for M_t for flasks 1 and 2, equation 1 becomes:

$$\frac{15.2 - M_r}{12.8 - M_r} = \frac{S_x + 0.78 \times 10^{15} + 8.0 \times 10^{13}}{S_x + 8.0 \times 10^{13}}$$

which becomes:

2. $$S_x = 40.8 \times 10^{14} - 3.25 \times 10^{14}\, M_r$$

and similarly using the data from flasks 2 and 3:

3. $$S_x = 35.8 \times 10^{15} - 3.46 \times 10^{15}\, M_r$$

Solving equations 2 and 3 simultaneously gives:

$S_x = 8.0 \times 10^{14}$ atoms, which is 1.33×10^{-9} moles
$M_r = 10.1\%$ [7]

From these and similar calculations, using data from all four flasks (even though that from flask 4 may not be entirely comparable), a value of between 10^{-5} and 10^{-6} is indicated for the molar concentration of the contaminating sulfur. This is comparable to the two estimates obtained by growth methods.

A second factor which would help to account for the lack of a ten-fold change in mutation rate between flasks comes from the relatively long half life of the S^{35}. During the 18 days of the experiment only one out of every 7.5 S^{35} atoms disintegrated, the remaining 6.5 acting as carrier.

Combining the contaminating (2×10^{-9} moles) and the untransmutated sulfurs (1.2×10^{-10} moles) then in flask 1 one could expect about nine out of every 1000 of the sulfur atoms to have actually transmutated during the time of the experiment. This would mean that only one atom out of 116 would have been capable of inducing mutations. In flask 2 the same number of S^{35} atoms must have transmuted but more S^{32} was added so only one atom out of 187 could have been trans-mutagenic. In flask 3 one atom out of 880 could have been trans-mutagenic. This gives an expected ratio of transmutation induced mutations of 100 in flask 1 to 62 in flask 2 to 13 in flask 3.

Although the observed mutation rates can be interpreted on the basis of radiation and transmutation as two separate causes of mutation, this is not necessarily the only possible explanation. First, it is possible that the stored sulfur present in the conidia of flasks 2, 3, and 4 might be present in such form that it acts as a protective agent against radiation effects (BARRON and DICKMAN 1949; FORSSBERG 1950). Second, it is possible that there was a more rapid utilization of sulfur from flask 1 than from the other flasks. This

[7] This is the highest value that any of our data gives for the percentage of radiation induced biochemical mutations. A value of about 6% is obtained when the data from other combinations of flasks are used and this fits well with the frequency found in radiation experiments.

would mean that the total radiation of the conidia in this flask might have been greater and yielded a higher mutation rate.

However, in addition to the fact that the frequency of mutation varies with the specific activity of the radioactive sulfur, another observation suggests that some factor other than radiation may be involved. The frequency of mutations observed here is considerably higher than is obtained in radiation experiments. Whereas TATUM *et al.* (1950) obtained mutation rates of 2.7% biochemical mutants with dosages of about 37,000 roentgens, here under conditions where the total dosage probably was not greater than 20,000 rep's we obtain over 15% biochemical mutants.

It is true that these experiments were done on growing (at least for part of the time) cultures which might influence the percentage of mutations recovered. That neither the low sulfur nor the growth conditions could cause such a high recovered mutation rate was shown by irradiating cultures on low sulfur at different times during growth to determine the relative recoveries of

TABLE 5

Effect of Growth on recovery of biochemical mutants

Flask	Distance from source	Time of exposure	Calculated dosage (r)	Number of isolates	Mutation frequency %
8	10 cm	After 24 hrs. of growth exposed for 5¼ hrs.	53,000	369	3.8%
9	60 cm	After 24 hrs. of growth exposed for 8 days	53,000	440	3.9%
10	10 cm	After 9 days of growth exposed for 5¼ hrs.	53,000	383	6.0%

mutations. Microconidia were inoculated onto 1 ml of low sulfur medium in each of three 25-ml flasks, which were then kept at 25°C for 24 hours. At this time it was apparent that the conidia had germinated but there was very little hyphal growth. The flasks were then treated in such a way that they all received the same total amount of radiation from a 100-curie cobalt-60 source, but given in one case intensively at the first of the growth period, in another slowly during the growth period, and in the third after the growth period. Table 5 gives the treatment and biochemical mutation rates obtained from these three cultures. It is apparent that the recovery of mutant types is reduced when growth occurs between the time of radiation and isolation of mutant strains and thus the rate of 15% is probably low.

If transmutation of S^{35} produces mutations, it might also be expected that it would produce only limited types of mutations. From the conversion of sulfur to chlorine, only genes in which sulfur occupies a key position would mutate. It should be possible to calculate the number of genes which would

have to contain sulfur in a key position to give rise to the number of mutations ascertained as arising from transmutation. Although we do not at present have adequate data to allow any confidence to be placed in the results of such a calculation, we will nevertheless follow a sample one through as an illustration. It is estimated that in flask 1, one sulfur atom out of 116 changed to chlorine during the experiment. Thus if there had been only one gene per nucleus which had an essential sulfur atom it would be expected that one mutation per 116 isolated cultures had been produced by transmutation. If we assume that 9% (the highest value that can be inferred from the data) of the mutants from flask 1 arose from transmutation, these would be expected to arise from 11 genes ($116 \times .09$). If there had been more genes that contained sulfur in a vital spot the mutation rate should have been higher. Of course, this calculation does not take into account any of the following types of gene-contained sulfur:

1. Sulfur which through transmutation produces nuclei which cannot survive, i.e., lethals.
2. Sulfur which even though lost does not produce a mutation.
3. Sulfur which induces nonbiochemical mutations. (Close to 100% of the isolates from flask 1 were morphological mutants.)
4. Sulfur which induces a second mutation in a nucleus already containing a biochemical mutation.

These would all tend to increase the value assigned to the number of sulfur-containing genes. If anywhere near correct, however, the small number is quite surprising as this would mean that all of the transmutation-induced biochemical mutations should be restricted to a few genes, and mutations of these should have recurred with a relatively high frequency. This is not apparent from an inspection of the types of mutations and it can be clearly determined only by a genetic analysis which is as yet incomplete.

There are several mutants whose nutritional needs are quite different from previously described radiation-induced types. These are amino acid mutants whose requirements are satisfied by any one of two or more different amino acids.[8] Of positively identified amino acid requiring mutants only 49 have growth requirements that are met by only one amino acid. The remaining 103 strains can be roughly divided into those (33) using compounds of the arginine-ornithine sequence (SRB and HOROWITZ 1944) and those (70) using leucine or one or more other amino acids. The former type is familiar in radiation work with Neurospora and in other biochemical studies, but the latter do not in general fit any of the currently accepted metabolic patterns. Many of these strains are satisfied by one or more of the sulfur-containing amino acids, methionine, cysteine and cystine, as alternatives to leucine. Additional compounds stimulating some of the strains in this group are isoleucine, valine,

[8] The possibility that our amino acid stocks were contaminated was tested by making paper chromatograms. Also, three of these strains were independently tested by DR. R. W. BARRATT, of Stanford University, whose results confirm ours.

phenylalanine, tyrosine and tryptophane. These stocks have not yet been tested to determine whether they are the result of single or multiple mutations but if they are multiple mutants, the problem would be even more complex. The other types of mutants (vitamin, yeast extract, etc.) show a distribution similar to that obtained from radiation experiments (BEADLE and TATUM 1945; TATUM, BARRATT, FRIES and BONNER 1950). It is quite possible that transmutations and radiations induce many of the same mutations. That they might be the same is not too unlikely since BARRON (1949) has shown that – SH groups are destroyed by radiation and this same group could certainly be destroyed by transmutation of the sulfur atom. If an – SH group gave specificity to a gene this gene would be changed by either irradiation or transmutation of the sulfur.

This pushes us toward the question: What are the active groups on genes? Is the phosphate group which is so common in nucleic acid an essential group or is it merely a part of the skeleton of the chromosome? The latter is suggested by the almost complete lack of a transmutation effect when radioactive phosphorus is used as a mutagen (RUBEN and STEINGLASS 1949). It is true that HERSHEY *et al.* (1951) found that phage is destroyed by the conversion of P^{32} into S^{32} but this killing may well have been due to effects other than mutations.

Regardless of the mechanism of its action, it is apparent that S^{35} is a very effective mutagenic agent, and in addition to being effective the techniques for using it are simple. It has a half life of 87 days which is long enough to permit storage in the laboratory for some time before decay has destroyed excessive amounts and yet its half life is short enough that disposal is not too difficult. The beta radiation that is given off is so weak that it is completely absorbed by a glass container and there is thus almost no danger to personnel. The isotope is inexpensive and obtainable in carrier-free form from Oak Ridge.

SUMMARY

The mutagenic effects of sulfur-35 have been tested on *Neurospora crassa*. The tests were conducted in such a way that the effect of the transmutation of S^{35} into Cl^{35} could be quantitatively distinguished from the radiation effect of the ionizing beta particle.

After 18 days of exposure to the S^{35} under growing conditions, microconidia were plated and individual colonies isolated. Tests for biochemical mutants showed that 15% of the conidia from the flask with the highest specific activity were biochemical mutants. Other flasks having successively lower specific activity gave 13%, 10% and 6% biochemical mutants. Calculations indicate that between 5% and 10% in each flask were radiation induced, the remainder presumably arising from transmutations.

Although carrier sulfur was added to separate flasks in ratios of 0, 10, 100 and 1000 parts per part of S^{35}, the actual ratios vary from this due to contaminating sulfur. The extent of this contaminating sulfur was estimated by three separate methods.

The types of mutations recovered appear to be much the same as would be expected from any radiation experiment with the exception of some of the amino acid requiring types. These unexpected types are characterized by their ability to grow on any one of a number of amino acid supplements.

A sample calculation has been made to determine from the experimental data the number of genes which could give rise to the amount of transmutation-induced mutation that is observed in these experiments. Although the data are inadequate to warrant any rigorous use being made of the calculation, it is interesting to note that it suggests that only a few genes may be involved. Conditions under which more genes than this might contain sulfur but fail to affect the result are discussed.

It is apparent from this work that sulfur-35 is a very potent and easily handled mutagenic agent for laboratory use, particularly with microorganisms.

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SEX COMPATIBILITY IN ESCHERICHIA COLI[1]

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GENETIC recombination in bacteria was first successfully studied in strain K-12 of *Escherichia coli* (TATUM and LEDERBERG 1947; LEDERBERG 1951). Since the nutritional mutants used in the crosses were derived directly from this strain under clonal propagation, their compatibility implied a homothallic or self-compatible sexual system (*cf.* WHITEHOUSE 1949). The inference that crossing was genetically unrestricted was supported by the absence of marked hereditary mating preferences among the segregants of a variety of crosses (LEDERBERG 1947, 1949; *cf.* LEUPOLD 1950). More recently, evidence has been secured for a system of sexual compatibility which was previously obscured by its unique inheritance via an infective agent.

METHODS AND CULTURES

Experimental techniques differed slightly in Madison and Milan, but the concordance of the results obviates a detailed comparison. Crossing procedures, genetic symbols and the detailed pedigrees of many stocks are given elsewhere (CAVALLI 1950; LEDERBERG 1947; LEDERBERG *et al.* 1951). Most of the experiments have involved crosses of the original K-12, of stocks clonally related to 58-161, a double mutant requiring biotin and methionine (B^-M^-), and stocks related to Y-10, a triple mutant requiring threonine, leucine, and thiamin ($T^-L^-Th^-$). (Since the original isolation of 58-161 and early crossing experiments (TATUM 1945; LEDERBERG 1947) its biotin requirement appears to have been lost. The methionineless stock is, fortunately, so stable that back mutations to prototrophy are undetectable under the conditions of crossing experiments.) Stocks carrying similar markers which have been extracted from crosses (LEDERBERG 1949; LEDERBERG *et al.* 1951; ROTHFELS 1952) will be designated *filial*.

Auxotrophic parents were crossed in the usual way, by plating washed suspensions into or on the surface of minimal agar to select for prototrophic recombinations. For example, 58-161 ($M^-T^+L^+Th^+$) cells plated with Y-10 ($M^+T^-L^-Th^-$) gave $M^+T^+L^+Th^+$ prototrophs in yields approximating one per million of the parents inoculated. The occurrence of prototrophs, thus

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selected, from platings of thoroughly investigated auxotroph parents has been taken as *prima facie* evidence of crossing, a conclusion supported by other experiments in several laboratories.

Prototrophic streptomycin-sensitive (S^s) stocks were crossed to S^r (streptomycin-resistant) auxotrophs by plating the parents on minimal-streptomycin agar. This selection for S^r prototrophic recombinants has been abbreviated *SRP*. To avoid confusion, some familiar expressions will be used in a restricted sense for this paper. Two stocks are termed *crossable* if the selection of recombinant genotypes is technically possible, that is, if they carry non-overlapping nutritional requirements, or if they are amenable to the *SRP* method. *Fertility* will refer to the experimentally observed production of recombinants from crossable parents. *Compatibility* will be reserved for the status of a stock with respect to the system to be described in this paper.

Most of the genetic studies with *E. coli* K-12 have involved the 58-161 (M^-) and the Y-10 ($T^-L^-Th^-$) lines, and other auxotrophs were used to a limited extent. All crossable stocks appeared to be fertile with each other, as expected in a homothallic system. It was not readily feasible, however, to test self-fertility, and in view of the common clonal origin of the various lines directly from K-12, this did not appear to be urgent. Occasional deviations in quantitative yields of prototrophs were ascribed to linkage relationships affecting the probability of detectable recombinations, and to secondary, perhaps indirect, modifiers of productivity in the test system.

RESULTS AND CONCLUSIONS

Self-incompatibility. The first evidence of a compatibility difference was recognized when a routine single-colony isolate, W-1607, of an M^- stock, unlike all others previously tested, was found to be completely infertile with "standard" $T^-L^-Th^-$ parents such as Y-10. At the time, W-1607 was not analyzed further, but in view of later work will be labelled F^-, in contrast to the type F^+. Although W-1607 was not fertile with any clonal derivative of Y-10 it was fertile with other auxotroph testers and with *filial* $T^-L^-Th^-$. Another M^- stock isolated after selection for resistance to nitrogen mustard was later recognized as a second recurrence of F^-.

In order to explain certain peculiarities in linkage behavior, it has been suggested that 58-161 and Y-10 differed from each other in chromosome structure, possibly as a result of the many kiloroentgens of X-rays to which they had been exposed in their history. To obtain crossable stocks which might be free of such differences, spontaneous prototrophic reversions from $T^-L^-Th^-$ (Y-10 line) to $T^+L^+Th^+$ were selected, factor by factor. Two such prototrophic reversions were secured, and were tested with $T^-L^-Th^-S^r$ (W-1177, a clonal derivative of Y-10) by the *SRP* method. Unlike the original K-12 or various other prototrophs from recombination experiments, the reversions proved to be completely infertile with W-1177. However, the reversion prototrophs were fully fertile with M^-S^r and with *filial* $T^-L^-S^r$ stocks in comparable *SRP* crossing tests, suggesting that the Y-10 line was

self-incompatible but cross-fertile. The similar compatibilities of the aberrant M^-, W-1607, and of the reversion prototrophs from the Y-10 line, when they were tested with a variety of K-12 derivative stocks made it apparent that W-1607 and the Y-10 line carried a similar " mutation," F^-.

Compatibility may be experimentally defined by the following properties:

$F^- \times F^-$	sterile
$F^+ \times F^-$	fertile
$F^+ \times F^+$	fertile

for which W-1607 and W-1177 serve as standard F^- testers. The application of this test (fertility in crosses × W-1607) to the ancestry of W-1177 has indicated that the F^- mutation in this line probably occurred during the isolation of T^-L^- (F^- strain, 679-680) from T^-L^+ (F^+ strain 679; TATUM 1945).

Test for an F^+ hormonal stimulus. The compatibility conditions may be summarized by the requirement that at least one parent must be F^+ to give a productive cross. This suggested that F^+ cells might secrete a hormone required for sexual reproduction. The following experiment was therefore set up to determine whether F^+ cells could stimulate an otherwise incompatible cross. $M^-Lac^+S^sF^+$ (58-161) was grown together with $M^-Lac^-S^rF^-$ (W-1607) in broth. The cell mixture was then crossed with $T^-L^-Th^-Lac^+S^sF^-$ (Y-10) and the prototrophs were characterized for *Lac* and *S*. All prototrophs from the only compatible pair, $M^-Lac^+S^sF^+ \times T^-L^-Th^-Lac^+S^sF^-$ should be uniformly Lac^+S^s. Prototrophs carrying either Lac^- or S^r markers would indicate an illegitimate crossing of $M^-Lac^-S^rF^- \times T^-L^-Th^-Lac^+S^sF^-$. These markers were, in fact, observed in about one fourth of the progeny, a large number of which must then have originated from the incompatible parents. In further experiments of similar design, it was shown that either of the F^- parents, W-1607 or W-1177, could be stimulated to cross with an otherwise incompatible partner by previous growth with F^+ cells. This stimulation occurred only when the F^- and F^+ cells were grown together prior to plating on minimal medium. Cell-free filtrates of F^+ or of mixed F^- with F^+ cultures were not effective, nor was the F^+ stimulus transmitted across a bacteria-tight filter through which the medium supporting F^+ and F^- cells was flushed from one compartment to another (DAVIS 1950a). At first sight, these experiments appeared to support the concept of an F^+ hormone, but without the substantiation of a cell-free preparation.

Inheritance of F^+/F^-. It has already been indicated that all *filial* stocks which have been tested have reacted as F^+, *i.e.*, no segregation of F^- has been observed in $F^- \times F^+$ crosses. This might be interpreted as an incident of linkage, a direct effect of sexual reproduction, or a form of non-Mendelian transmission. As a partial discriminatory test, the exceptional prototrophs of the previous experiment were examined. These presumably issued from $F^- \times F^-$ under the influence of an F^+ stimulus, and might be expected to inherit the F^- quality, but when a number of such prototrophs were crossed by the *SRP* method to F^- testers, all proved to be compatible. The " hormonal " influence

of F^+ cells in the stimulation experiments was therefore inherited by the presumed $F^- \times F^-$ progeny. The large proportion of exceptional prototrophs suggested the feasibility of a direct test of the parents.

Transmission of F^+. The same marked pairs of F^- and F^+ stocks used in the stimulation experiments were grown together in the same way. Each mixed culture was then streaked out on EMB lactose or streptomycin agar to re-extract the component that was originally F^-. Single colonies were then propagated in broth, and the cultures crossed to F^- testers. The cultures were also re-checked for purity, especially to rule out contaminating F^+ cells. In every experiment, well over half of the originally F^- cells had become F^+; in some instances, all of the colonies tested. In a further check of purity, the prototrophs in the test crosses were found to carry no other markers from the original F^+ donor. The new F^+ cultures retained this transduced trait through numerous single colony isolations, and appear to be as stable as the standard F^+. F^+ was also repeatedly transmitted in series to F^- carriers. Each of a number of F^+ and F^- pairs tested behaved in the same way. No evidence of the transduction of any other markers was observed in these experiments.

This transmission is remarkably efficient. Lac^+F^+ and Lac^-F^- cells were mixed in broth at an initial concentration of 10^8 per ml. each. After one hour at 37°C the mixture was streaked out on EMB lactose agar. Individual Lac^- colonies were tested; about 10 percent had become F^+ during this brief exposure. Mixtures in saline or minimal medium, or held in broth at 4°C for one hour evinced no F^+ transductions. Cultures grown together overnight in synthetic medium manifested only a limited degree of transduction. Whether prototrophs from $F^- \times F^+$ crosses become uniformly F^+ by a more efficient route than inter-cellular transduction remains to be determined.

In contrast to the efficiency of intact cultures, cell-free preparations have so far been inactive. Following ordinary, by no means exhaustive, centrifugation, the sedimented cells of F^+ cultures have retained all of the F^+ transducing activity, and left none in the supernatant. Other preparations from F^+ cultures that have given negative or inconclusive results include autolysates, penicillin lysates, sonic lysates, aqueous extracts of dried and alumina-ground cells, and heat-inactivated cells. Further kinetic studies have been hampered by technical difficulties in testing the compatibility of large numbers of isolates.

In many experiments, it is desirable or necessary to test the transduction of F^+ without spending the time and effort required to reisolate the exposed clones. For such experiments, a *filial* $M^-T^-L^-F^+$ stock has been isolated as a segregant from a persistent heterozygote M^-/T^-L^- (LEDERBERG 1949). This stock can function as a source of F^+ in combinations with M^- or $T^-L^-Th^-$ parents without itself participating in effective recombinations, owing to the overlapping nutritional requirements which prevent the development of prototroph offspring. That is to say, it can be used as an otherwise inert source of the F^+ agent for these experiments. It should be feasible to study the kinetics of F^+ transduction by direct measurements of the yield of

prototrophs from crosses of M^-F^- with $T^-L^-Th^-F^-$ cells that have been cultured with $M^-T^-L^-F^+$ far varying intervals of time. This possibility is based upon two findings: 1) platings of washed cells of $M^-T^-L^-F^+$ with an incompatible cross on minimal agar gave no prototrophs, presumably owing to the limited transduction of F^+ under these conditions, as previously demonstrated, and 2) platings of $T^-L^-Th^-F^-$ with washed cells of a mixture of M^-F^- and $M^-T^-L^-F^+$ that had been held in broth for one to two hours gave considerable yields of prototrophs. The latter seem to show that F^+ is phenotypically effective immediately after its transfer, but it must be admitted that only the first step is blocked on minimal agar, and that the subsequent development of the compatibility phenotype may proceed on the crossing plates.

Relationship of the F^+ agent to latent virus. The infective transmission of F^+ at once calls to mind the latent bacterial viruses, especially since strain K-12 itself is lysogenic for such a phage, λ. However, the filtrability of bacteriophage at once differentiates it from the F^+ agent, as well as from the gametes themselves. A re-examination of existing stocks at once showed no correlation between lysogenicity and F^+. Nonlysogenic as well as lysogenic stocks are freely intercrossable (E. M. LEDERBERG 1951). In further experiments, F^+ was readily transduced from a nonlysogenic, λ-sensitive donor to F^- stocks, whether lysogenic or not. Finally, the transmission of λ and of F^+ from a lysogenic F^+ to a sensitive F^- occurred independently and separately to give derived λ^+F^- and λ^-F^+ stocks. The cell-to-cell transmission of F^+ in short term experiments is far more efficient than that of λ. These agents share the property of infectious transmission, but there is no evidence that λ is either a determinant or an agent of genetic recombination, and it is sharply delineated from F^+ in many other respects.

An incompatibility phenocopy. During the early investigation of genetic recombination it was noted that crosses of 58-161 (M^-F^+) and Y-10 ($T^-L^-Th^-F^-$) that had been grown to high densities (*ca.* 10^{10} cells per ml) with the help of vigorous aeration were markedly unproductive. It has now been shown that this low fertility is related to incompatibility. Aerated cultures of 58-161 (genetically M^-F^+) behave phenotypically as if they were F^-, giving few or no prototrophs with F^- testers, while remaining fertile with the same testers transduced to F^+. This behavior is a "phenocopy"; several dozen clones isolated from these suspensions, but re-grown without aeration, behaved again as F^+.

The physiological basis of the phenocopy is poorly understood. The substitution of CO_2 or N_2 for air or oxygen abolished the development of the phenocopy, but did not reverse it in fully grown suspensions. The modification also occurred when cells were aerated at 26°, although the lower temperature reduced the growth rate (crudely estimated) to the level of unaerated cultures at 37°. Cells harvested from partly grown aerated cultures at low cell density were phenotypically F^+, although the inoculum had been a previously aerated phenocopy. When the cells later reached saturation density, they again reacted as F^-. The effects were similar whether aeration was accomplished by bub-

bling air (Madison) or rolling the cultures (Milan). An accumulated metabolite is possibly involved, but has not yet been directly demonstrated.

Only those cultures derived from 58-161 have responded to aeration. The reaction is inherent in the cells and not in the F^+ agent, as determined by the responses of stocks made up from F^- cells and F^+ agents transduced from various sources. In particular, Y-10 transduced by F^+ from 58-161 has not shown any definite response to aeration. Aeration might be thought to affect the F^+ agent itself, or to interfere at some other stage of the development of the compatibility phenotype. Most of the limited evidence supports the latter view. As already mentioned, aeration does not seem to influence the genetic stability of the F^+ potentiality, as might be expected if it were inactivating the F^+ agent, and the response to aeration is an attribute of the residual genotype, rather than of the transmissible F^+ agent. In addition, aerated 58-161 cells which were phenotypically F^- successfully transmitted the F^+ agent to W-1607 in mixtures plated out after a short time.

Hfr (*High frequency of recombination*). In the course of selection experiments for resistance to nitrogen mustard (CAVALLI and VISCONTI 1948), a derivative of 58-161 was isolated which displayed a high frequency of recombination and was designated *Hfr* (CAVALLI 1950). M^-Hfr crosses with $T^-L^-Th^-F^-$ appear to produce one hundred to one thousand times as many prototrophs as M^-F^+ crosses, but a proper comparison of mating efficiency requires a detailed kinetic analysis (NELSON 1951) which remains to be applied here.

In compatibility tests, *Hfr* resembles the F^+ phenotype. However, efficient attempts to demonstrate the transduction of an F^+ agent from *Hfr* to F^- stocks have been entirely unsuccessful. Similarly, all progeny of $Hfr \times F^-$ have been F^- (with very rare exceptions). These progeny resemble the F^- parent in every material respect; they are compatible with F^+ testers, but not with F^-; they fail to transduce F^+ to standard F^- testers; they can accept an F^+ agent from standard F^+ stocks to become compatible. The progeny of $Hfr \times F^+$ are usually F^+.

An F^+ agent may be present in the *Hfr* stocks in a masked or bound form. One old stock of *Hfr* was observed to have lost its exceptional productivity. It proved to have become F^+, as judged both by mating efficiency and by the capacity to transmit the agent.

The *Hfr* behavior affords another instance of the separability of the compatibility phenotype and the transmissible F^+ agent.

It has been suggested (*cf.* HAYES 1952) that the F^+ agent acts directly a a vehicle of genetic recombination. Since the propagation of the F^+ agent persists in incompatible phenotypes (aerated 58-161) and conversely recombination occurs without the transmission of an F^+ agent to the progeny ($Hfr \times F^-$), this hypothesis may be inadequate to account for all of the evidence. We suggest instead that the F^+ agent is one causal element which determines sexual compatibility in conjunction with the residual genotype and environmental conditions.

Compatibility preferences. The infective transmission of F^+ permits a com-

parison of $F^+ \times F^-$ with $F^+ \times F^+$ against a uniform residual genotype. In all the combinations tested: $M^- \times T^-L^-Th^-$, $M^- \times P^-G^-$ (requiring proline and glycine, W-1678), and $T^-L^-Th^- \times P^-G^-$ plated on minimal agar, $F^+ \times F^+$ has been less productive than the corresponding $F^- \times F^+$. The effect has differed in different combinations, being most striking in $T^-L^-Th^- \times P^-G^-$, where the $F^- \times F^+$ cross has been more than one hundred times as productive as $F^+ \times F^+$. Unfortunately, no $P^-G^-F^-$ is yet available for the reverse cross. The differential is also apparent (about a tenfold increment) in comparisons of $T^-L^-Th^-F^+ \times$ aerated and unaerated M^-F^+ (58-161). In this instance, the F^- status is phenotypically impressed rather than genetically determined. These results are readily compatible with the concept of relative sexuality (HARTMANN 1929). F^- would have a grade of zero, 58-161 (unaerated) would be 2^+, Y-10F^+ ($T^-L^-Th^-F^+$) would be 3^+, W-1678 ($P^-G^-F^+$) would be 4^+, and *Hfr* the highest $^+$. The fertility of different combinations would be proportional to the difference in their relative grade. Although a number of stocks so far tested have agreed with this scale, many more must be tested and the productivities of different crosses must be quantitatively expressed to provide a basis for final judgment. It cannot be said whether differences in grade stem from the nutritional mutations directly, or from other genetic differences inherent in the various stocks and not otherwise detectable.

Compatibility and linkage. The limitations that are imposed on linkage analysis by the necessity for selective isolations of sexual progeny have been discussed at length elsewhere (LEDERBERG *et al.* 1951). Nevertheless, the linear arrangement of genes in *E. coli*, K-12 has strong experimental support (LEDERBERG 1947; ROTHFELS 1952). The most specific evidence of deviations from the familiar pattern of chromosomal segregation is the structure of persistent heterozygous diploids, which occur in certain crosses (LEDERBERG 1949, 1951). As a rule these diploids are heterozygous for all of the differential markers of the parents except for *S* and *Mal* (streptomycin response, maltose fermentation). The latter has been proved to be hemizygous in the otherwise heterozygous diploid, from which it has been concluded that the diploids are regularly deficient for a chromosomal segment which includes the linked *Mal* and *S* markers. This deficiency also perturbs the segregation of other markers which although digenic are adjacent to the segmental deficiency. If one assumes that a similar deficiency is the rule in the ordinary, transient zygotes, one must expect that normal linkage relationships will be obscured not only for *Mal* and *S*, but for other markers in accordance with their proximity, exactly as has been found for *Mtl* and *Xyl* (mannitol, xylose fermentation) (NEWCOMBE and NYHOLM 1950; CAVALLI 1950; LEDERBERG *et al.* 1951). Although deficiency for the *Mal-S* segment occurs with great regularity, diploids have been isolated which are heterozygous for each one of the ten or more differential markers of the parents, including *Mal* and *S*. These intact diploids, although rare, are compelling evidence that the underlying chromosomal mechanisms in *E. coli* are regular although complicated by modifications not yet thoroughly understood.

A hitherto enigmatic question has been the determination of which parental genotype would suffer the segmental elimination. Some regularities had been observed previously: that is, in crosses that would now be symbolized $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^-$ the diploids were usually hemizygous Mal^-. When it was observed that a *filial* (*i.e.*, F^+) $T^-L^-Th^-Mal^-$ parent gave the opposite result, hemizygous Mal^+, in a comparable cross, this was taken as evidence for structural heterozygosity of the original parents. These differences are also reflected in the distribution of markers to haploid prototroph recombinants. The detailed segregation differences between parental (F^-) and *filial* (F^+) $T^-L^-Th^- \times M^-$ cited in a previous paper (LEDERBERG *et al.* 1951) are reproducible with $T^-L^-Th^-$ made F^+ by transduction. These experiments are therefore immaterial to the cited hypothesis of structural difference.

These crosses have been studied more closely since the compatibility situation was better understood. $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^-$ gives diploids which are mostly hemizygous Mal^-. $M^-Mal^+F^+ \times T^-L^-Th^-Mal^-F^+$ gives diploids mostly hemizygous Mal^+. Unfortunately, material is not available for diploid analysis of a comparable cross $M^-Mal^+F^- \times T^-L^-Th^-Mal^-F^+$. If one accepts the higher F^+ potency of the $T^-L^-Th^-Mal^-F^+$ over $M^-Mal^+F^+$, as postulated earlier on the basis of productivity comparisons, we can generalize that segmental elimination tends to occur in the contribution of the parent with the higher relative potency. This is borne out by the segregations in haploid prototrophs, comparing the various combinations: A, $M^-F^+ \times T^-L^-Th^-F^-$; B, $M^-F^+ \times T^-L^-Th^-F^+$; and C, $M^-F^- \times T^-L^-Th^-F^+$. (For more elaborate tabulations, see LEDERBERG *et al.* 1951. Cross A is given in table 5A, Cross B in 5B, and Cross C gives segregations indistinguishable from B. Further documentation and discussion is held in abeyance pending a substantiation of the concept of relative potency.)

Occurrence of the F^+ agent. Some forty new strains of *E. coli* have been found that are fertile with K-12 testers. These have been screened from over 2000 separate isolates by means of *SRP* selection. Unfortunately, W-1177, F^-, was used as the tester for most of the screening experiments, so that potentially fertile strains which behave as F^- were passed over. A re-screening of a limited number of *E. coli* strains with the use of an F^+ tester has produced a crop of F^- fertile strains about equal to the number of fertile strains detected with W-1177 as a tester (DOROTHY C. GOSTING, unpublished). In at least seven new strains, F^+ has been detected by its transduction to W-1607 and W-1177, but some of the foreign agents appear to be unstable in the K-12 line. One other new strain that is compatible but F^- by the criterion of transduction has been infected with the F^+ agent from K-12. However, its mating behavior was not altered, and the latent F^+ agent could be detected only by its re-transfer back to W-1607. In view of this diversity of behavior, and possible biasses in the collection of this sample of cross-fertile strains, no generalizations can be stated as to the role of the F^+/F^- compatibility system in strains other than K-12.

Salmonella typhimurium and a number of infertile strains of *E. coli* have

been tested for $F+$ with negative results by the criterion of transduction. However, the "Waksman" strain of *E. coli,* ATCC 9637 (DAVIS 1950b) which had previously been thought to be infertile, carries an $F+$ agent. Upon reexamination, this strain was found to be self- and inter-fertile (with K-12), but at a low rate which barely permitted an unequivocal demonstration. The extremely low productivity of this strain must be ascribed to other, unknown causes, and not a deficiency with respect to the compatibility factor.

DISCUSSION

The inference of a sexual mechanism in *E. coli* has rested almost entirely on indirect genetic evidence. No one has succeeded so far in verifying or controverting this conclusion by a morphological demonstration of the biology of genetic exchange. Efforts in this direction had been discouraged by the apparently sporadic occurrence of such exchanges in mixed cultures. In an unanalyzed homothallic system, the rare occurrence of sexual fusions must be ascribed to chance. With a probability as low as one per million that a given pair of cells would mate, there is little likelihood of successful and verifiable microscopic observations. For this reason, the research of the past five years has been devoted largely to the exploitation of cross-breeding for physiological and formal genetic analysis, rather than to studies on the sexual mechanism itself.

The present studies have opened an avenue for further work depending on physiological and genetic control of mating. Hitherto, morphological studies were based on the concept of homothallism. One would have anticipated that sexual fusions occurred equally among the cells of a given parental culture and in cross-matings. Possibly relevant morphological processes that occurred uniquely in a mixed culture, and not in the parents alone might have been overlooked. The more recent recognition of self-incompatibilities necessitates a reversal of this point of view. Studies on the morphology of self-incompatible cultures are now seen to provide a necessary control on observations in mixed cultures where the results of a sexual process are genetically detectable. In addition, it is now and should become more and more feasible to plan experiments on a rational basis to provide the maximum frequencies of sexual fusions. It may therefore be hoped that this limitation to microscopic verification will be overcome.

Despite these hopeful auguries, the incompatibility experiments have raised more questions than they have answered. The physical basis of the transduction of the $F+$ agent is especially paradoxical, for the effect is transmitted from one cell to another, and in heredity, with very high efficiency, but no evidence of a material agent in transit has been secured. It is possible, of course, that the ambulant factor is highly unstable, or that it is communicated by direct contact. Some irony may also be seen in the fact that the one ambulatory gene (excluding λ from such consideration) to be detected in *E. coli* so far is decisive for the entire process of sexual recombination, and that its extra-nuclear transmission was responsible for its having been overlooked. By

contrast, each of the genetic factors of *Salmonella typhimurium* appears to be transducible by means of phage particles (ZINDER and LEDERBERG 1952). The Salmonella system differs from *E. coli* not only in the filtrability of the agent of genetic exchange, but in the individual transmission of each factor, no linkages of any kind having been detected. As discussed elsewhere, however, the fragmentation of the genotype in transduction does not necessarily exclude a chromosomal basis for normal hereditary transmission (LEDERBERG 1952). Further work may be expected to clear up the now obscure relationships of the distinctive processes of recombination now known for different bacteria, sexual recombination involving genetic linkage groups, and transduction of individual genes or very small fragments.

The F^+/F^- relationships suggest speculations on heterogametic differentiation. Evidence supporting a physiological differentiation of F^+ and F^- parents is provided by HAYES' experiments (1952) which imply that streptomycin-"killed" cells can function as F^+ but not as F^-. Morphological correlates of this differentiation have not been demonstrated. The effects of this differentiation on the segregation mechanism are still *sub judice*, but analogies are not difficult to find in the higher organisms, *e.g.*, in the selective elimination of paternal sex chromosomes in *Sciara coprophila* (METZ 1938).

Whether the incompatibility relationships are evidence of a rigorously heterothallic system remains to be settled by further work. The decision rests on the interpretation of $F^+ \times F^+$ crosses. If cells of identical F^+ status are actually compatible with each other, we should not speak of heterothallism, but rather of a genetic requirement for fertility. The low productivity of many $F^+ \times F^+$ crosses is the main argument for a more complex situation. We have seen, however, that the compatibility phenotype of 58-161 (F^+) is subject to modification by environmental effects. One might then argue that, to different degrees, F^+ cultures are heterogeneous with respect to compatibility phenotype. On this basis, crosses of $F^+ \times F^+$ cultures may actually involve $F^+ \times F^-$ cells. Differences in apparent potency might then refer to the fractions of phenotypic variants. Alternatively, F^+ may exhibit different grades of expression, depending on the genotype of the culture and on the environment. Although the latter interpretation seems more likely, a definite conclusion is not yet possible, and we are not even entitled to insist on the premise that an effective opposition of F^+ to F^- is a prerequisite of mating.

SUMMARY

Fertility of *E. coli* crosses has previously been thought to be homothallic or genetically unrestricted. This view has been altered with the discovery of self-incompatible stocks, designated as F^- mutations. Thus, $F^- \times F^-$ is completely infertile. $F^- \times F^+$ and $F^+ \times F^+$ are both fertile, but the latter combination is less productive in such a way as to suggest a gradient of relative sexual potencies among various F^+ stocks.

Self-compatibility is determined by an ambulatory or infective hereditary factor that is readily transduced from F^+ to F^- cells in mixed culture. The

phenotypic expression of F^+ is subject to environmental control (aeration) in some stocks.

The polarity of crosses with respect to compatibility status influences the segregation mechanism in an orderly way, not yet well understood, but interpretable on the basis of a sexual process underlying recombination in *E. coli*.

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